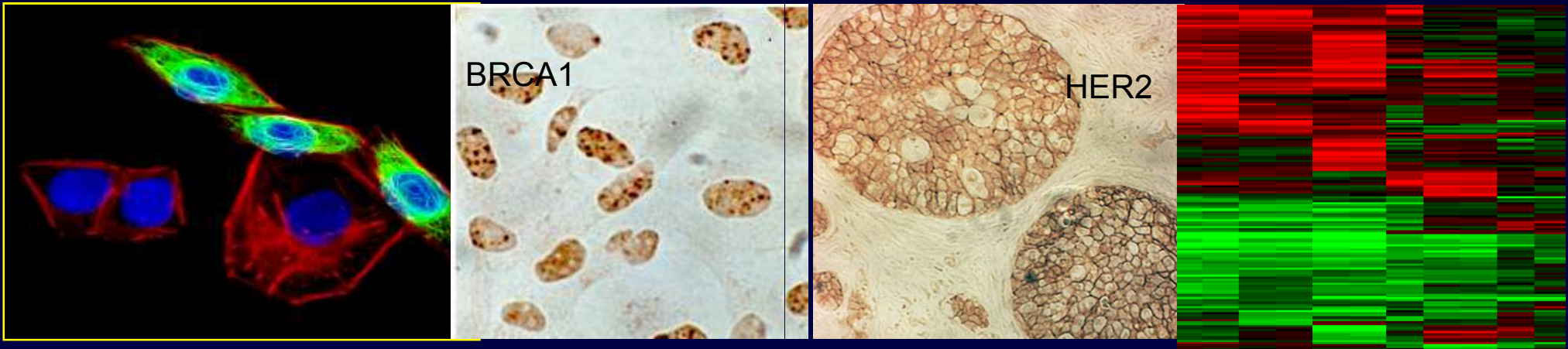
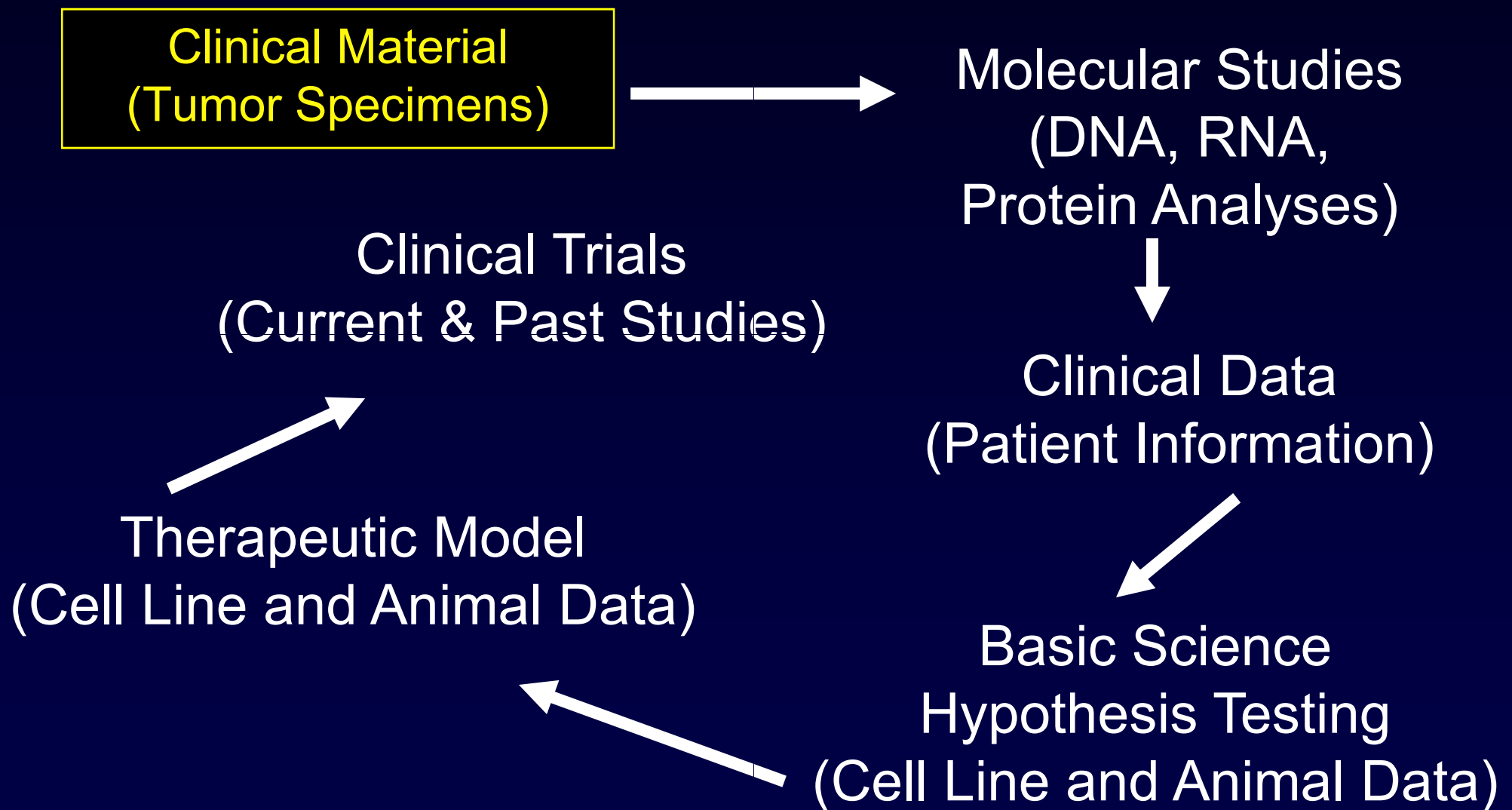


Molecular Diversity of Human Breast Cancers:

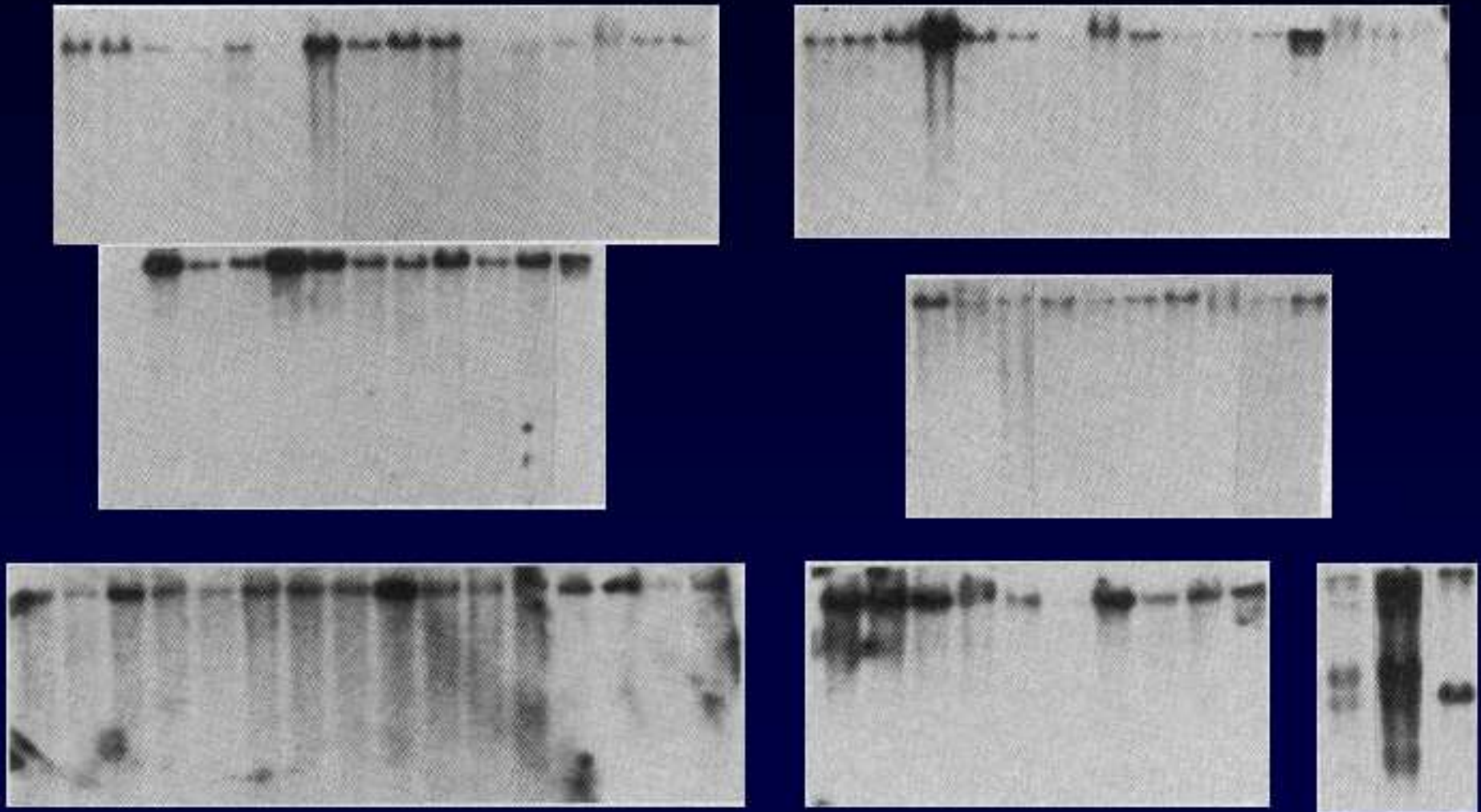
Biologic and Therapeutic Implications



HER-2/neu Program at UCLA

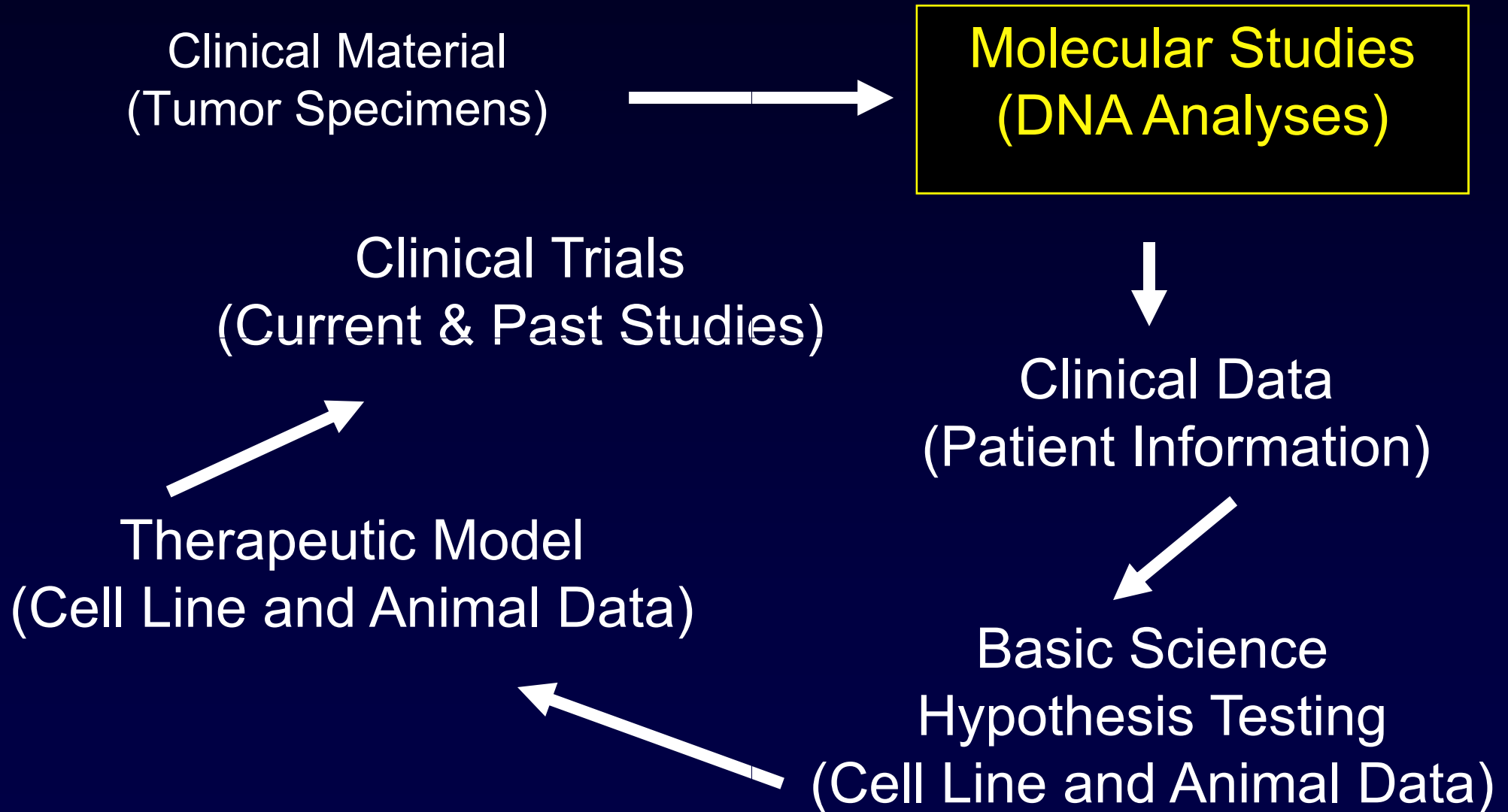


HER-2/*neu* Gene is Amplified in Human Breast Cancers

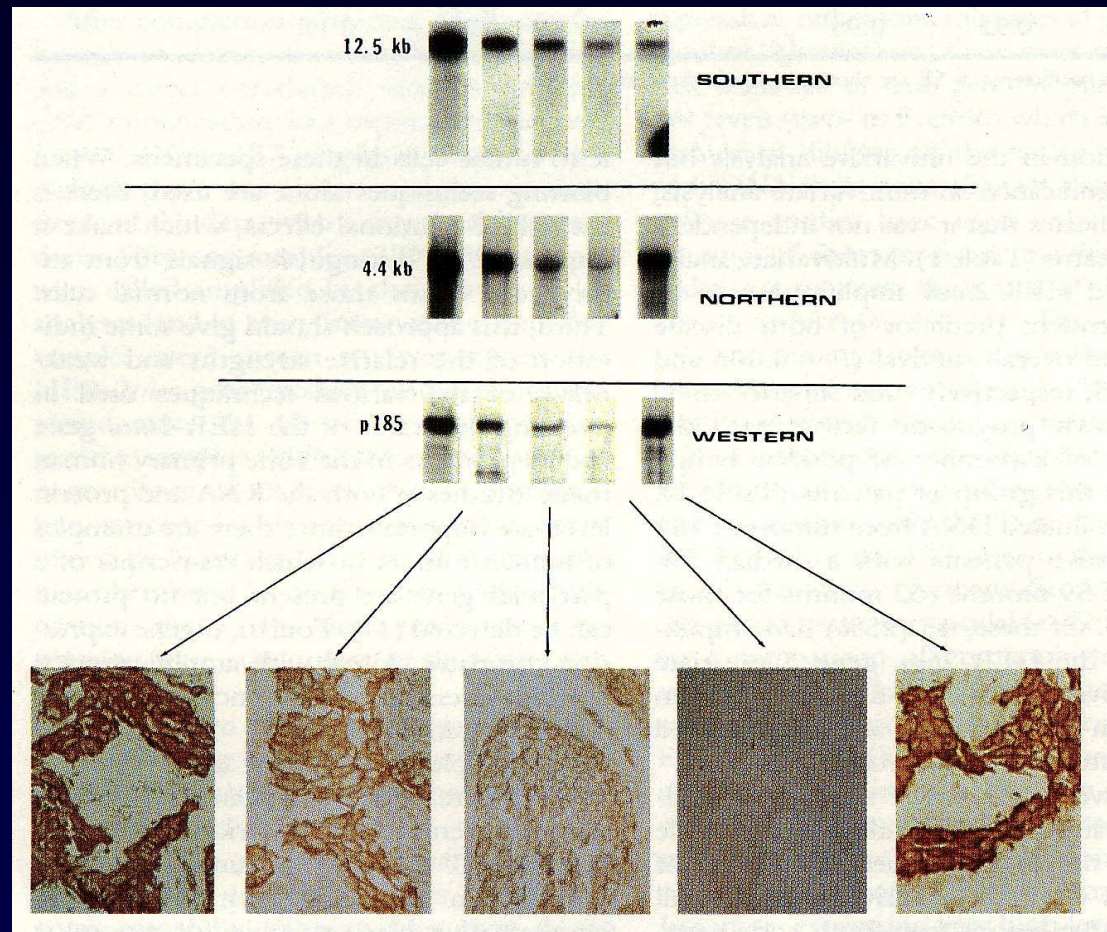


Slamon DJ, et al. *Science* 235: 177-182, 1987

HER-2/neu Program at UCLA



The HER2 Alteration

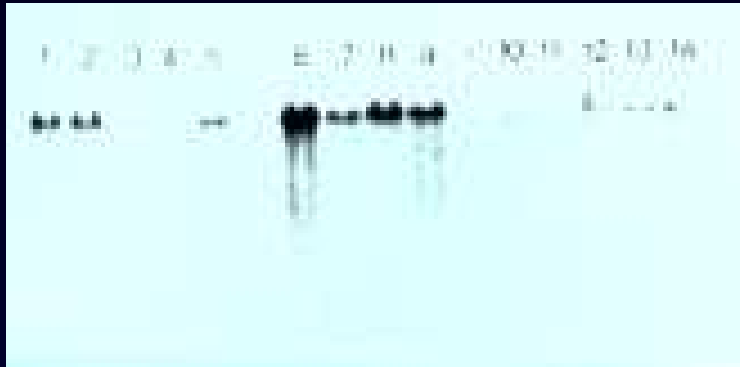


Southern

Northern

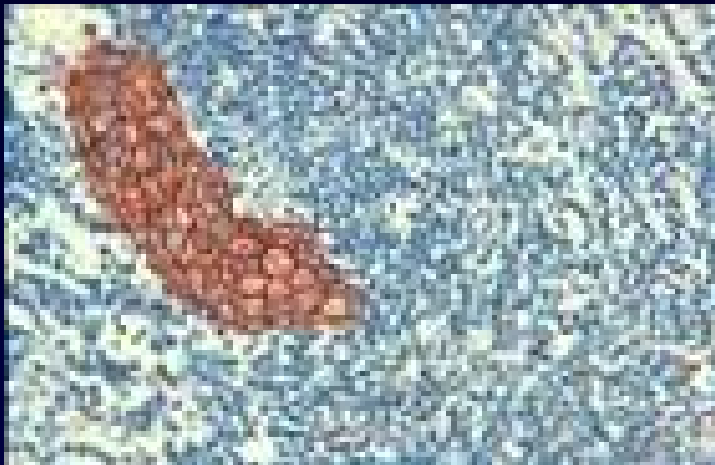
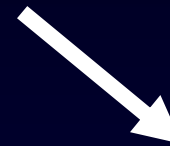
Western

IHC

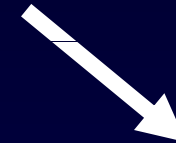


HER-2 Oncogene
Amplification

Breast Cancer



HER-2 Oncoprotein
Overexpression



Shortened Survival

Median Survival from First Diagnosis

HER-2 overexpressing	3 yrs
HER-2 normal	6 - 7 yrs

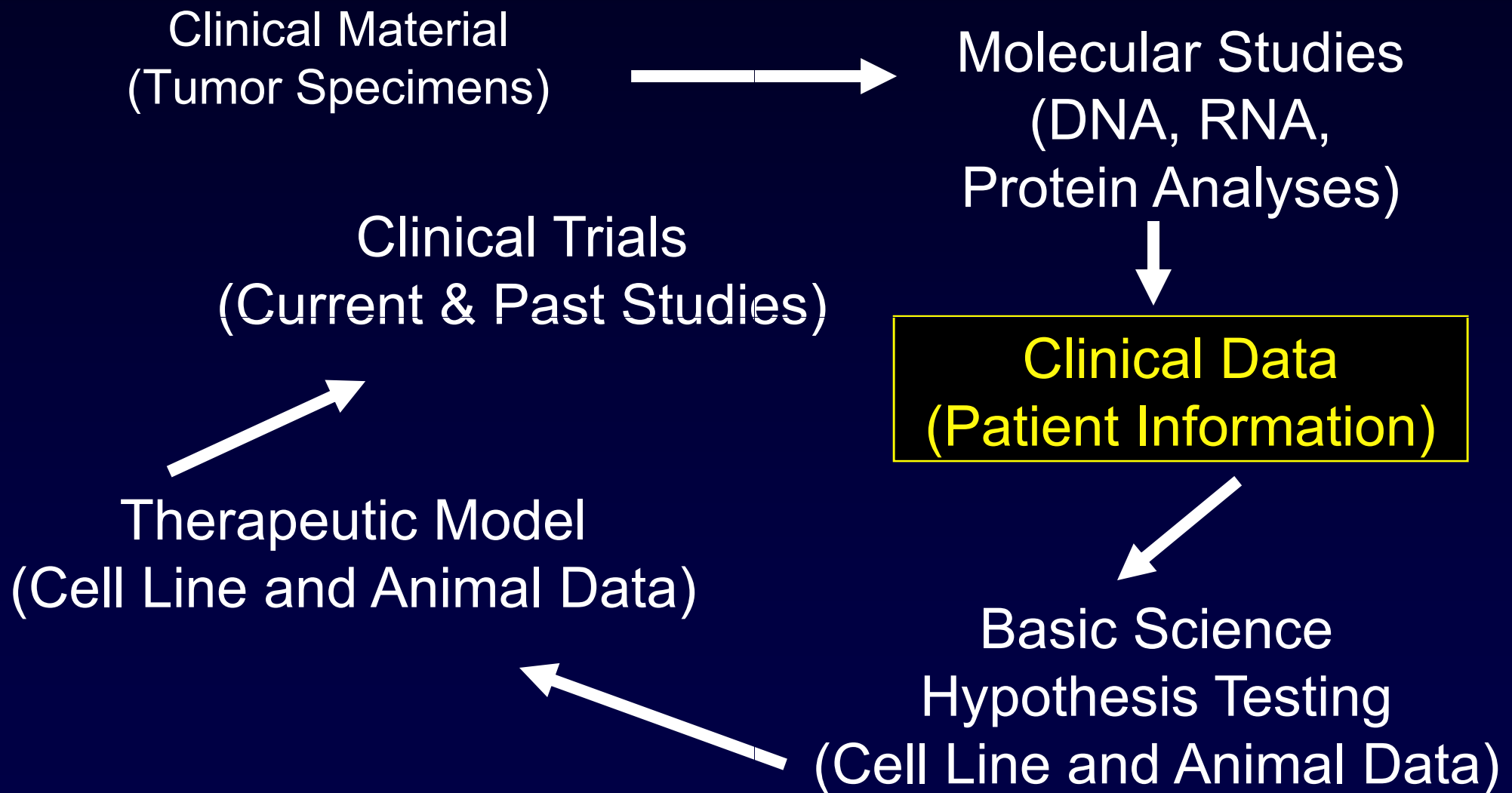
Median Survival from First Diagnosis in a Node-Positive Cohort

- ◆ Median survival in the HER2 normal (non-amplified) cohort = 6.8 years
- ◆ Median survival in the HER2 amplified cohort = <3 years
- ◆ HER2 amplification was an independent prognostic variable in multi-variate analyses using all standard prognostic variables

CONTROVERSIES

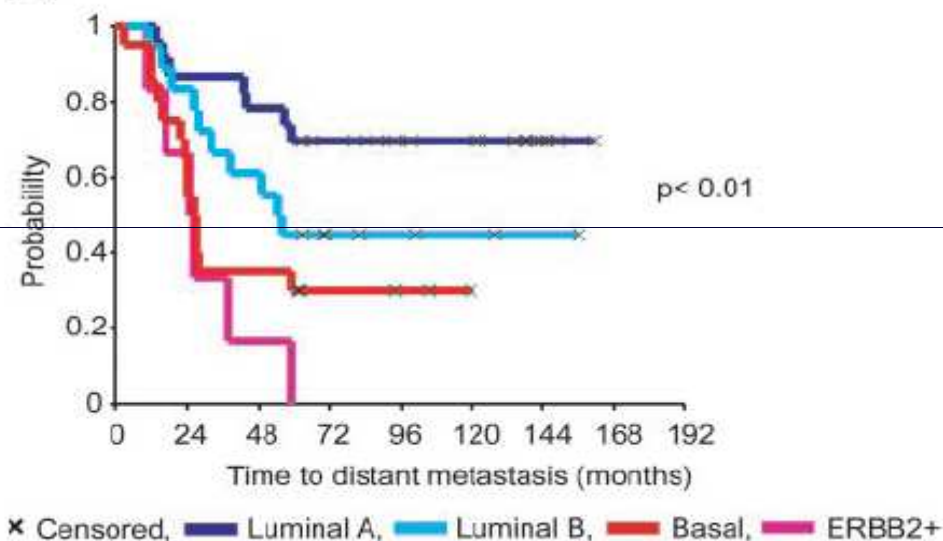
- ◆ It is NOT amplified at a rate of ~25% but much less frequently (~10-15%)
- ◆ There is no association between amplification and clinical outcome

HER-2/neu Program at UCLA



Breast Cancer Subtypes are associated with disease outcome

A van't Veer data set



B Norway/Stanford data set

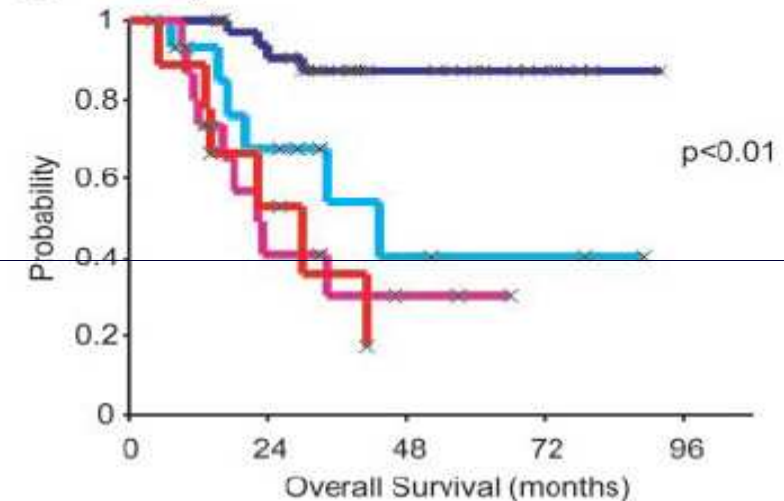
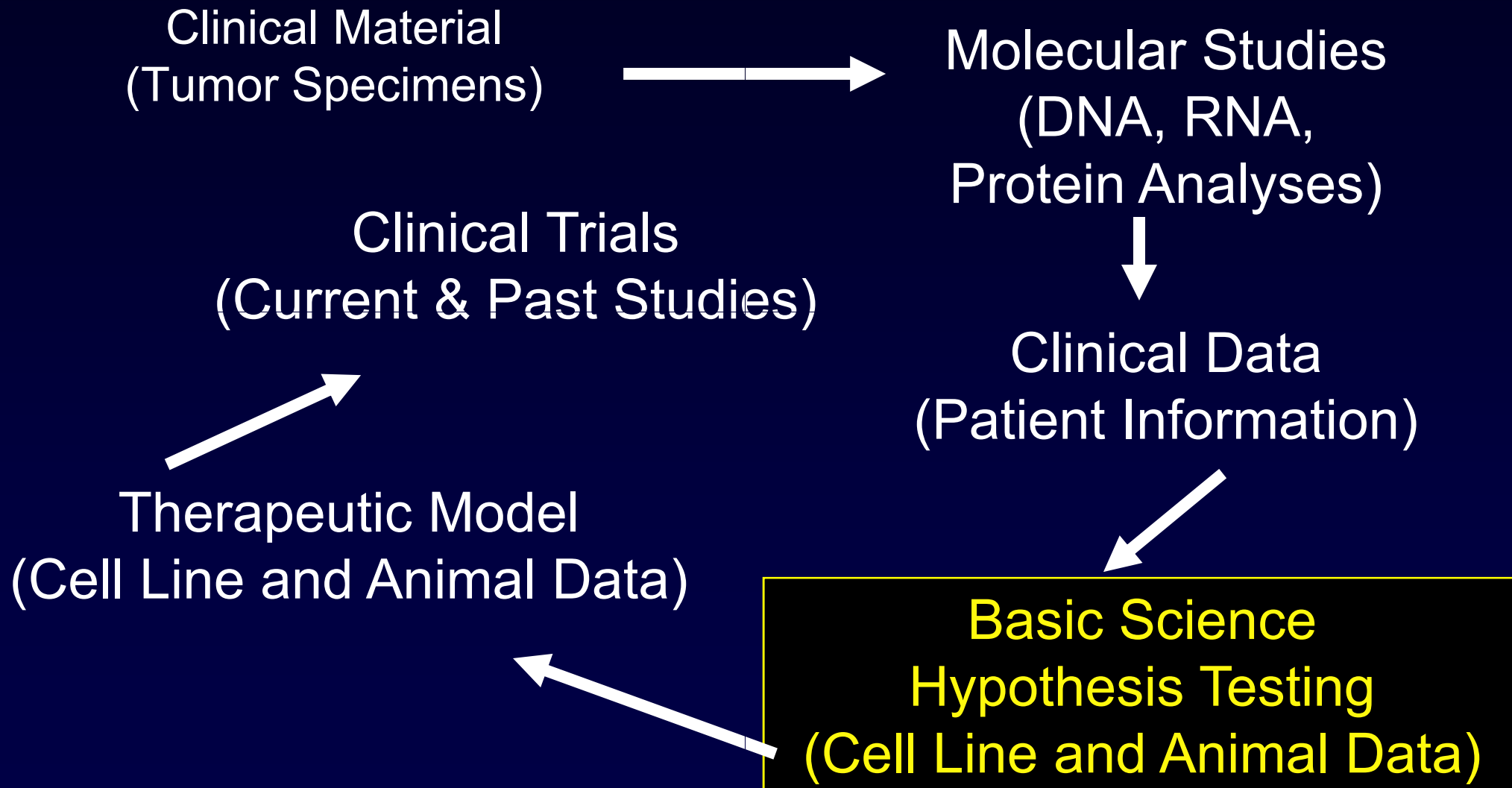


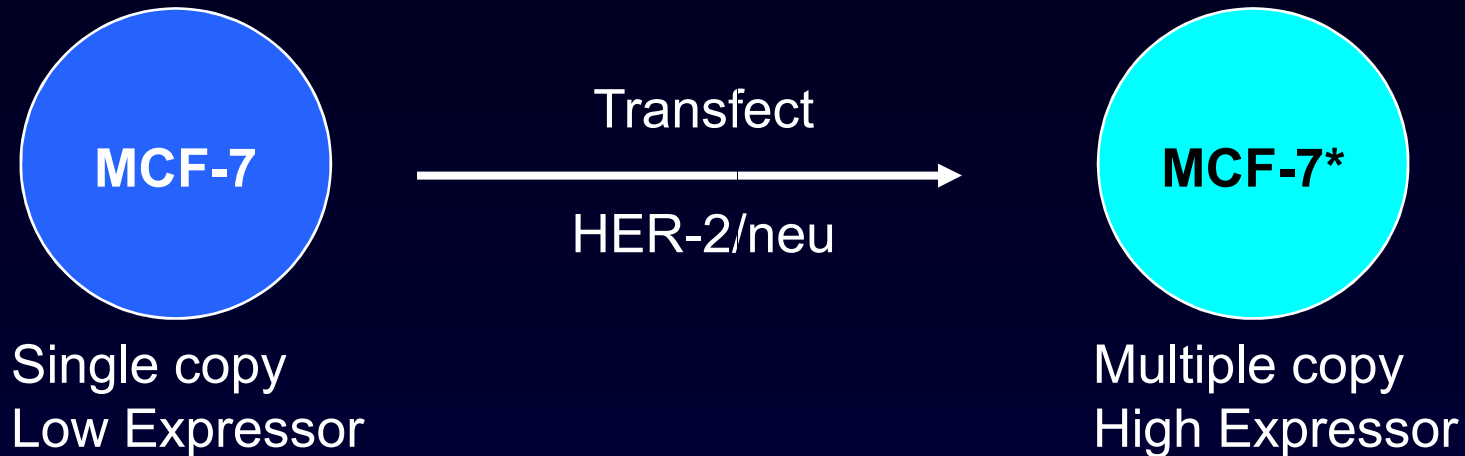
Fig. 5. Kaplan-Meier analysis of disease outcome in two patient cohorts. (A) Time to development of distant metastasis in the 97 sporadic cases from van't Veer *et al.* Patients were stratified according to the subtypes as shown in Fig. 2B. (B) Overall survival for 72 patients with locally advanced breast cancer in the Norway cohort. The normal-like tumor subgroups were omitted from both data sets in this analysis.

HER-2/neu Program at UCLA

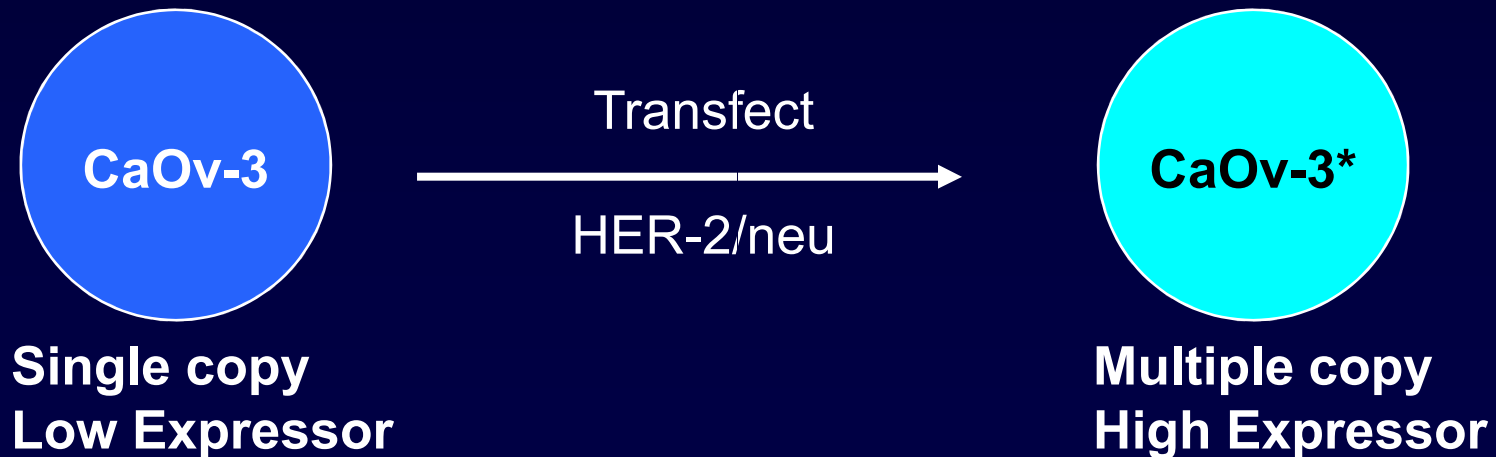


Target Validation - A

Human Breast Cancer Cells



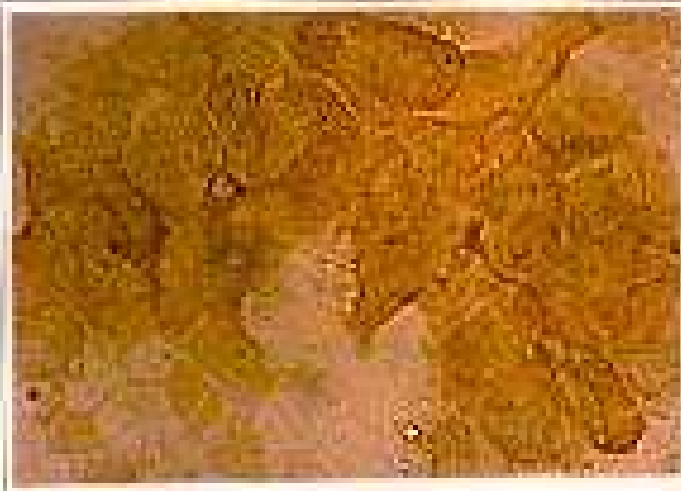
Human Ovarian Cancer Cells



***Consistent results in 9 additional Breast & Ovarian Cancer Cell Lines**

Immunohistochemistry

MCF 7

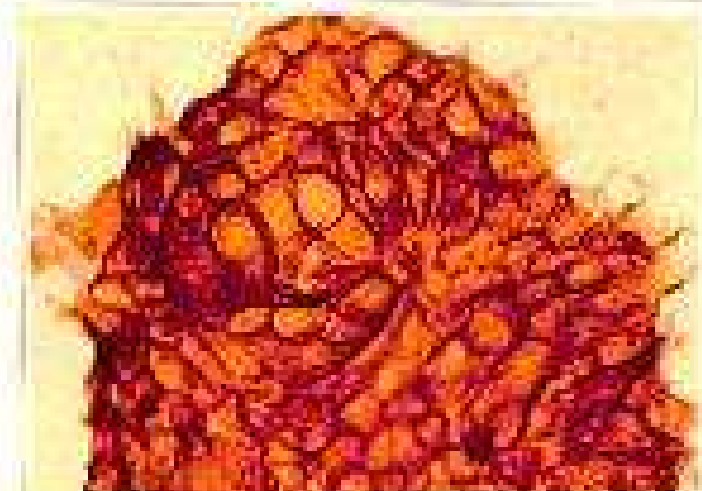
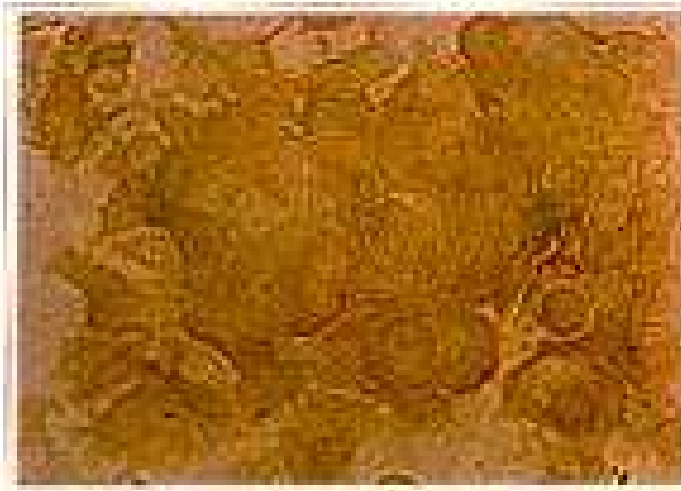


+ Control



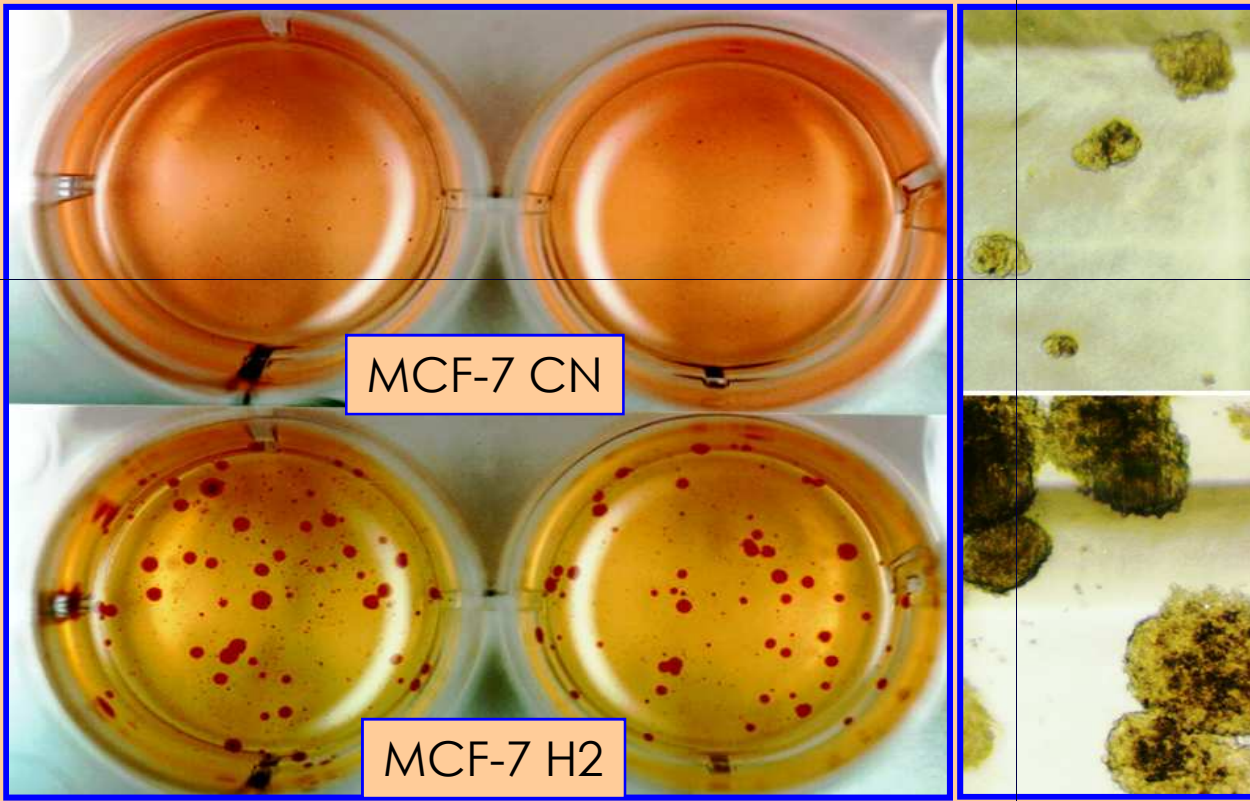
+ HER-2/neu

CaOV 3

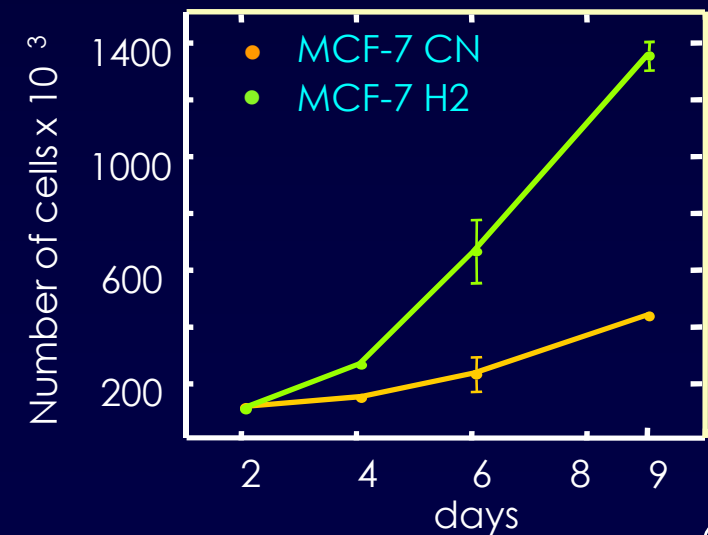
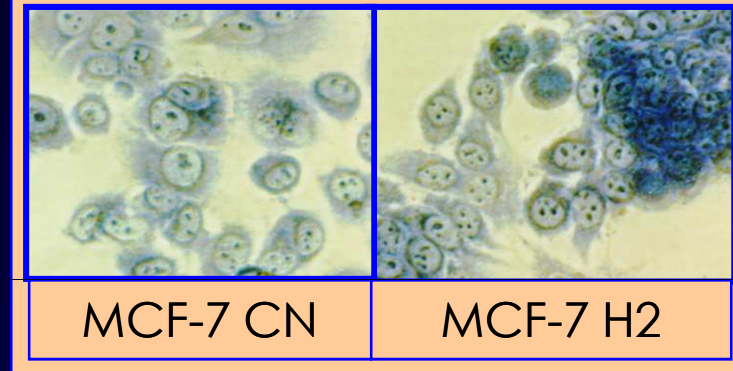


Engineered HER-2 Over-expression in MCF-7 cells Increased Proliferation and Decreased Contact Inhibition

Anchorage-Independent Growth

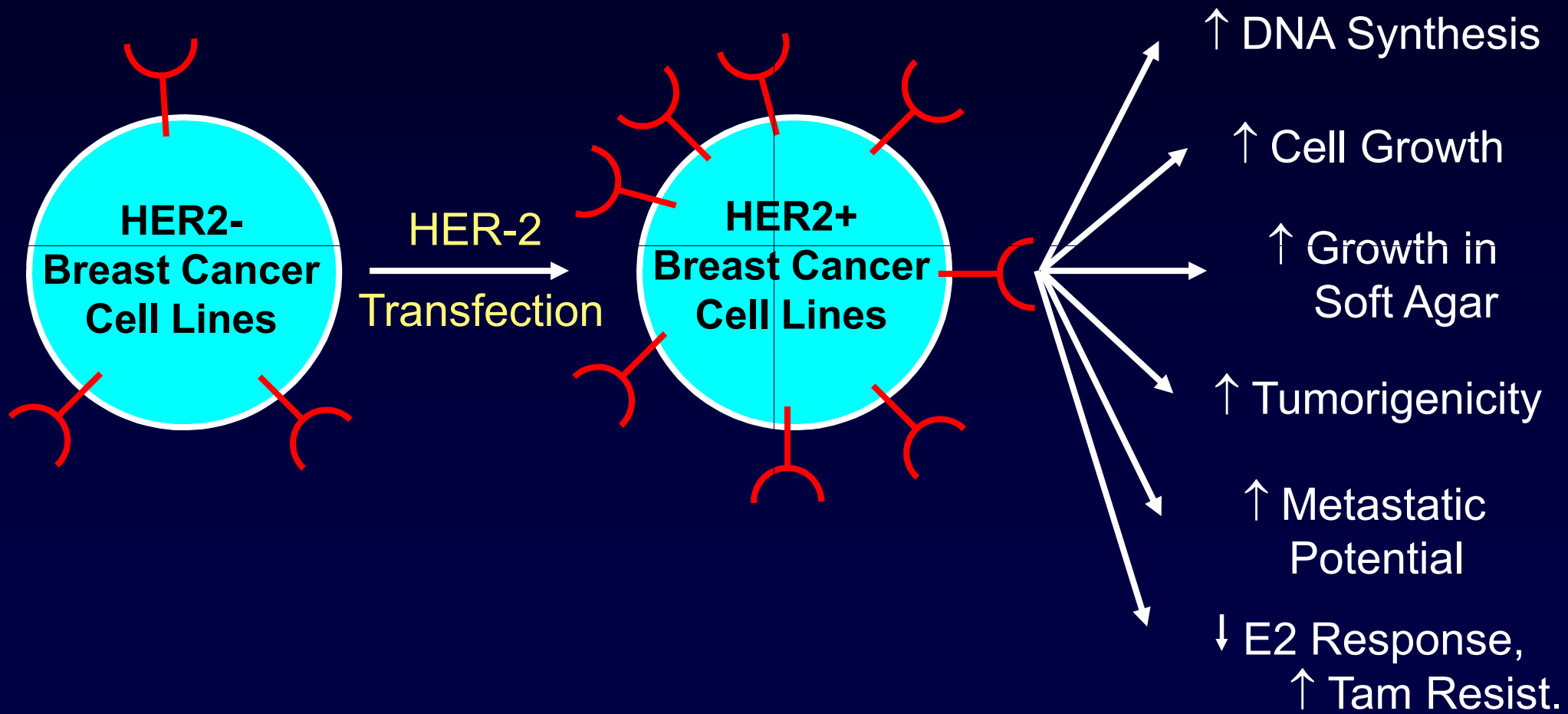


Growth on Plastic





Biologic Effects of HER-2/*neu* Overexpression in Human Breast Cancer Cells



HER-2/neu Program at UCLA

Clinical Material
(Tumor Specimens)



Molecular Studies
(DNA, RNA,
Protein Analyses)



Clinical Data
(Patient Information)



Basic Science
Hypothesis Testing
(Cell Line and Animal Data)



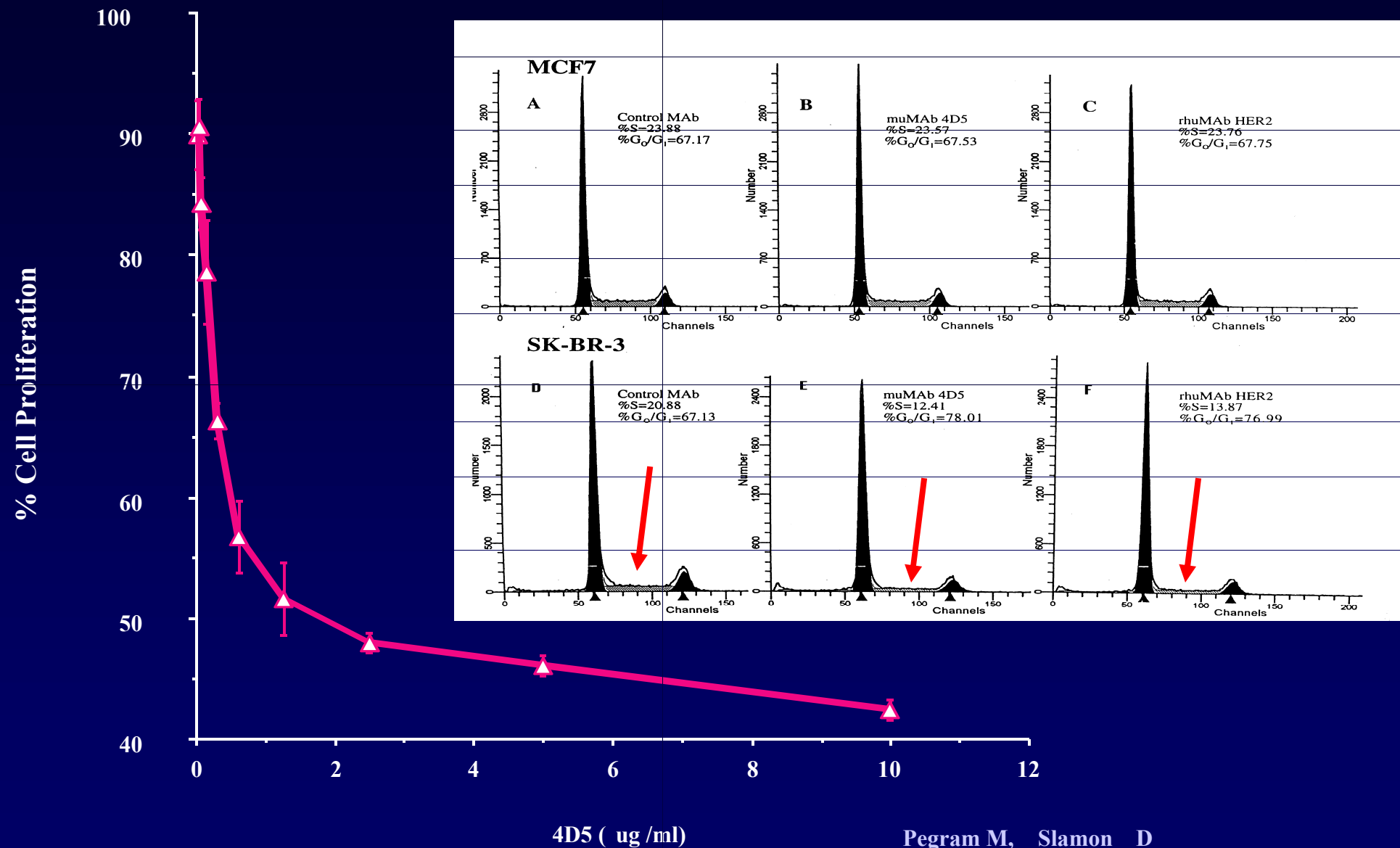
**Therapeutic Model
(Cell Line and Animal Data)**



Clinical Trials
(Current & Past Studies)

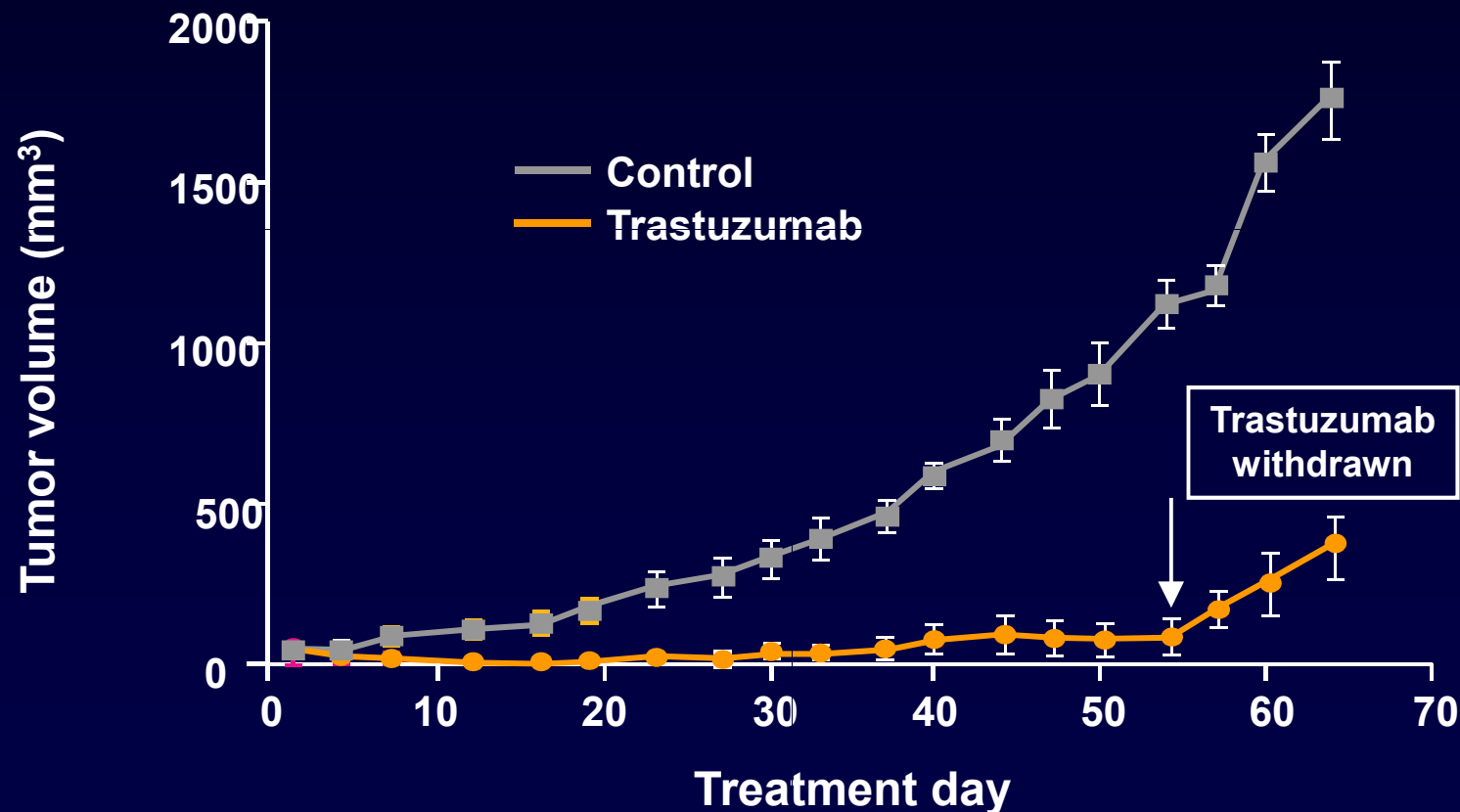
Target Validation - B

Dose-dependent anti-proliferative effects of 4D5 against HER2-overexpressing breast carcinoma cells *in vitro*

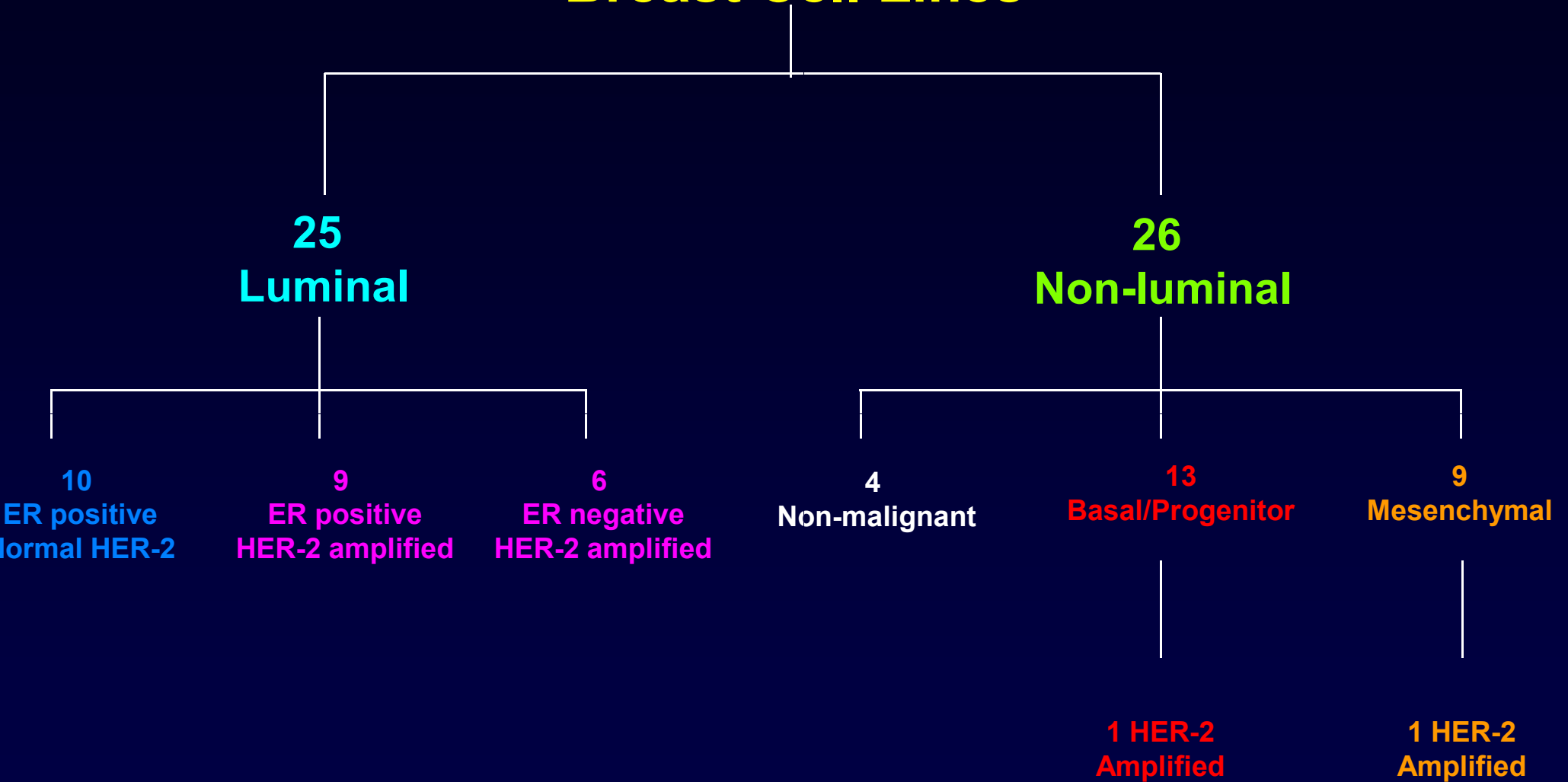


Preclinical Impact of Trastuzumab on Tumor Growth

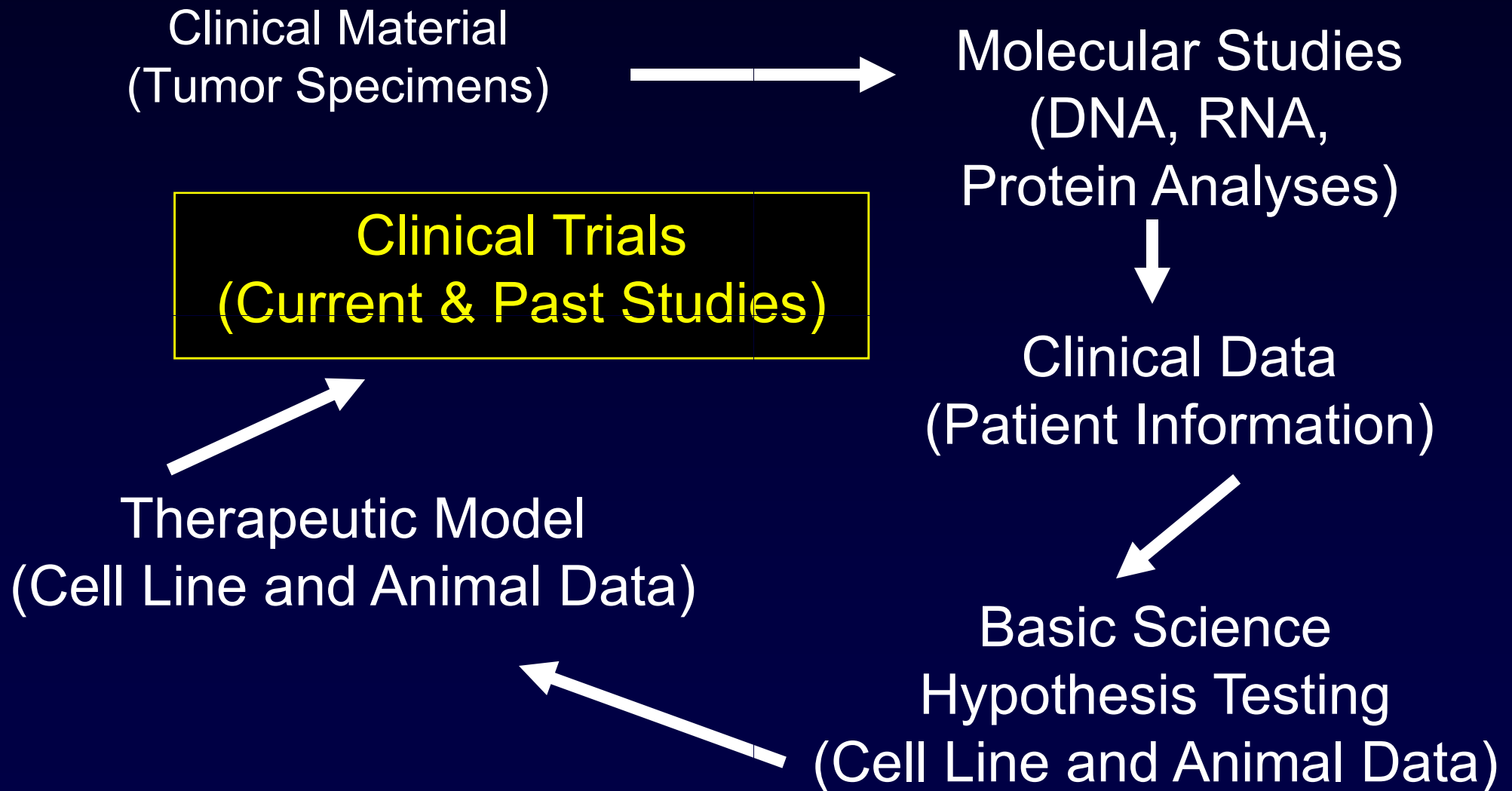
Effect of Trastuzumab Treatment on HER2+ Breast Cancer Xenografts



51 Human Breast Cell Lines



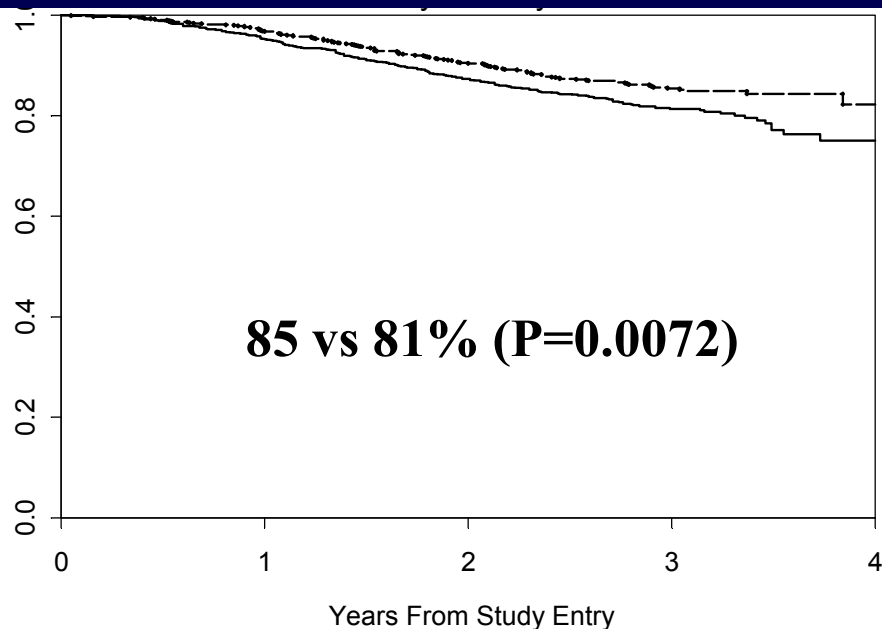
HER-2/neu Program at UCLA



CALGB 9741

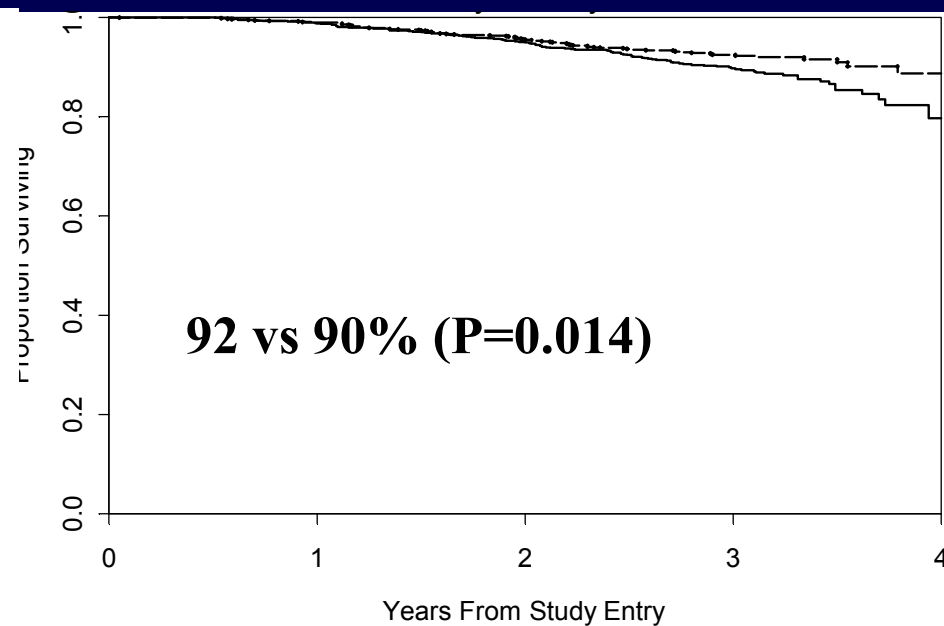
Interim Analyses

Disease-Free Survival



--- q 2 wks N= 988 Events= 136
— q 3 wks N= 985 Events= 179

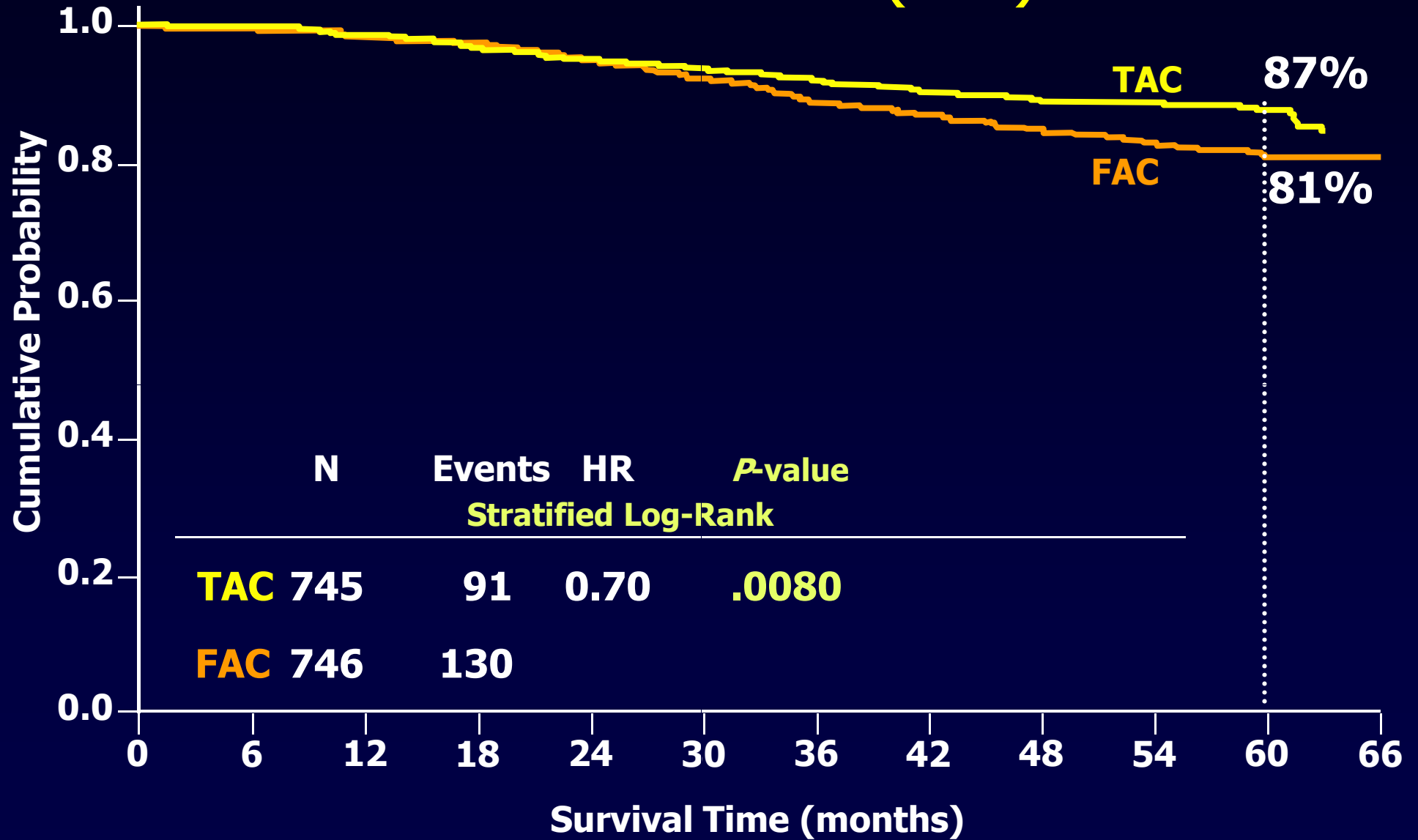
Overall Survival



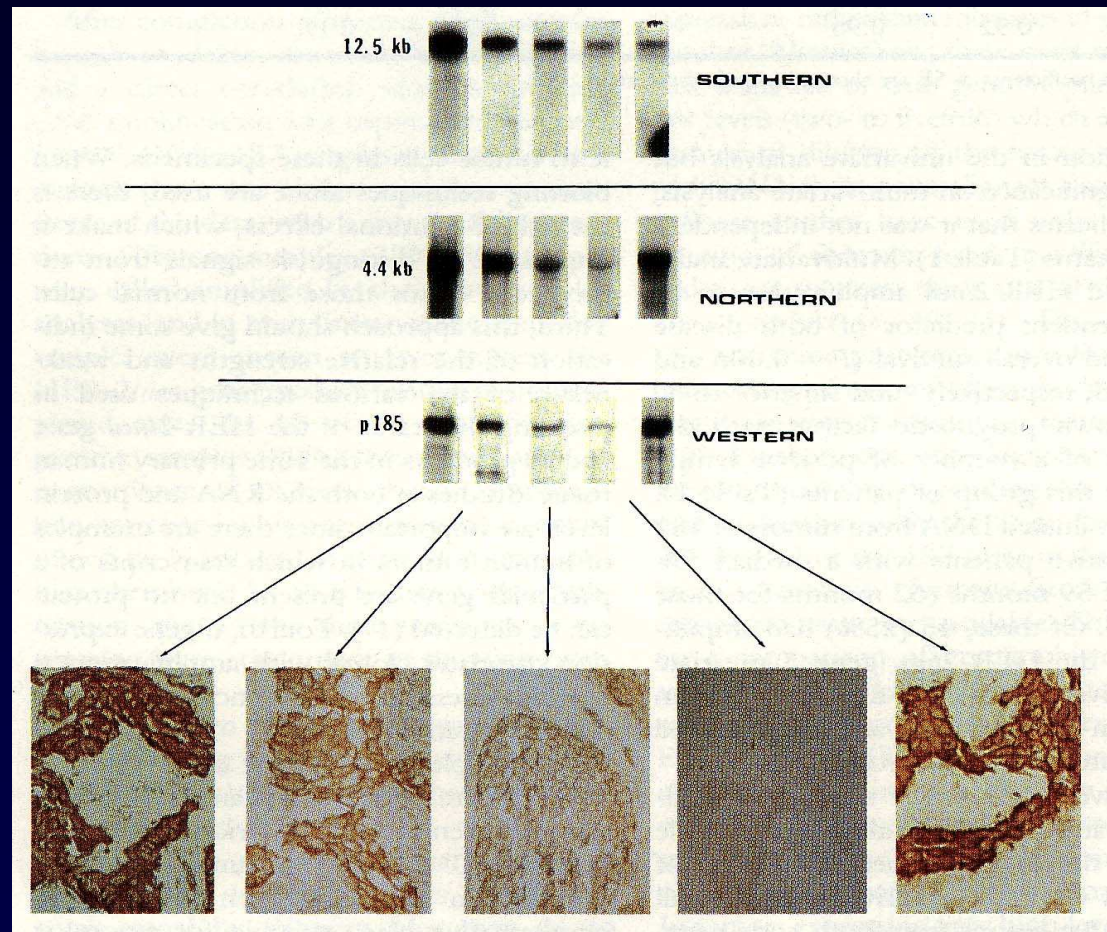
--- q 2 wks N= 988 Events= 75
— q 3 wks N= 985 Events= 107

N = 1973; Median F/U = 36 mos

Overall Survival (ITT)



The HER2 Alteration



Southern

Northern

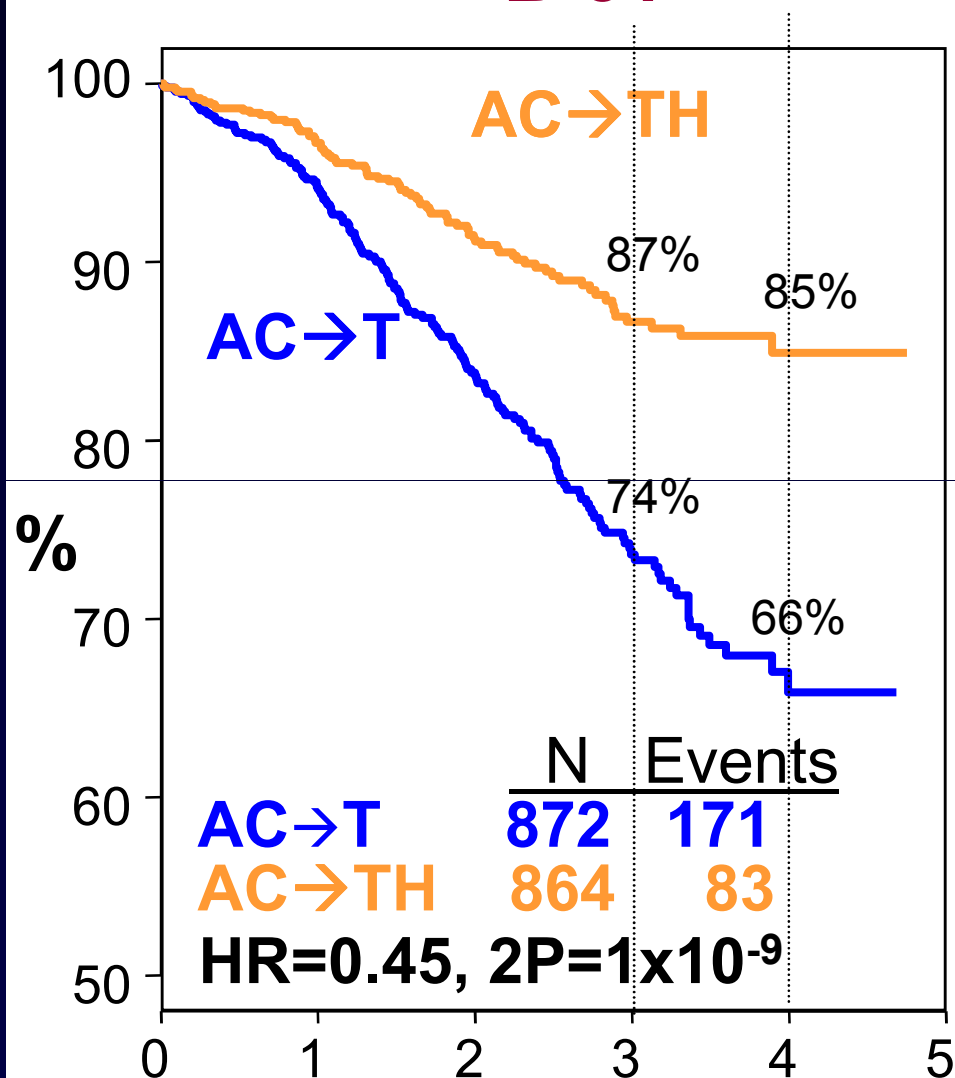
Western

IHC

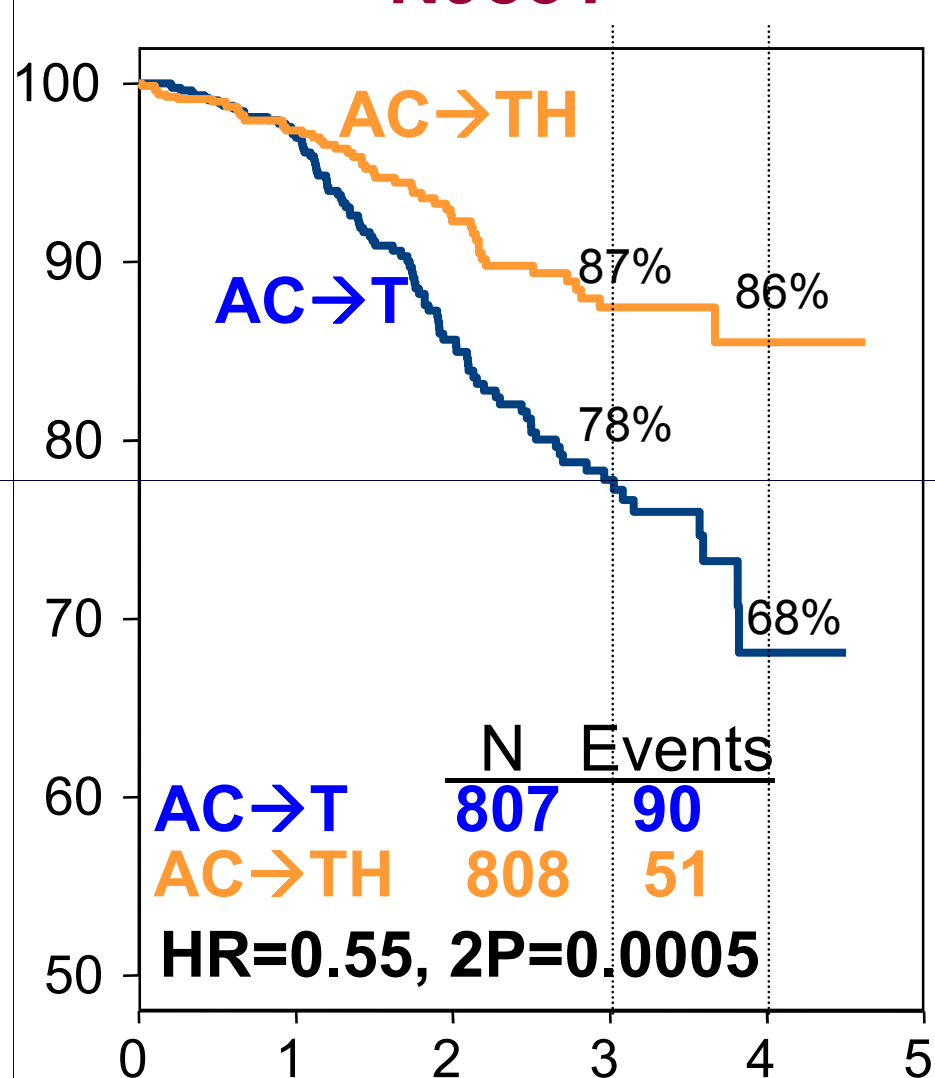
Slamon et al. *Science* 1989

Disease-Free Survival

B-31



N9831

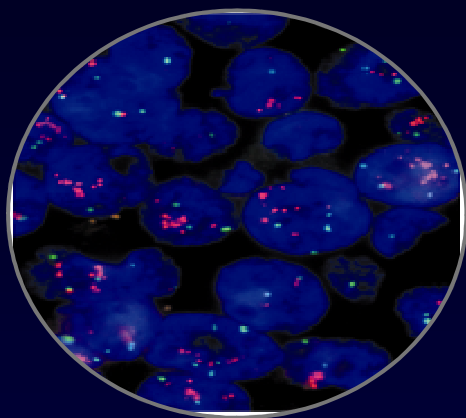


Lessons from the HER2 Story

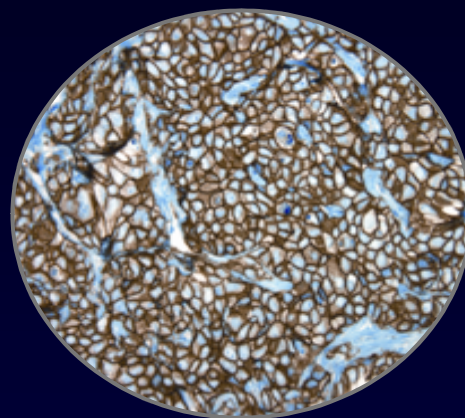
- ◆ 1.) Target Identification
- ◆ 2.) Target Validation
- ◆ 3.) Preclinical Confirmation
- ◆ 4.) Determination of Potential Usage Preclinically
- ◆ 5.) Clinical Translation - Proof of Concept
- ◆ 6.) Clinical Optimization

Clinical Significance of HER2

Testing of Primary Breast Cancers



HER2 gene
amplification (FISH)



HER2 protein
overexpression (IHC)

Why test for HER2?

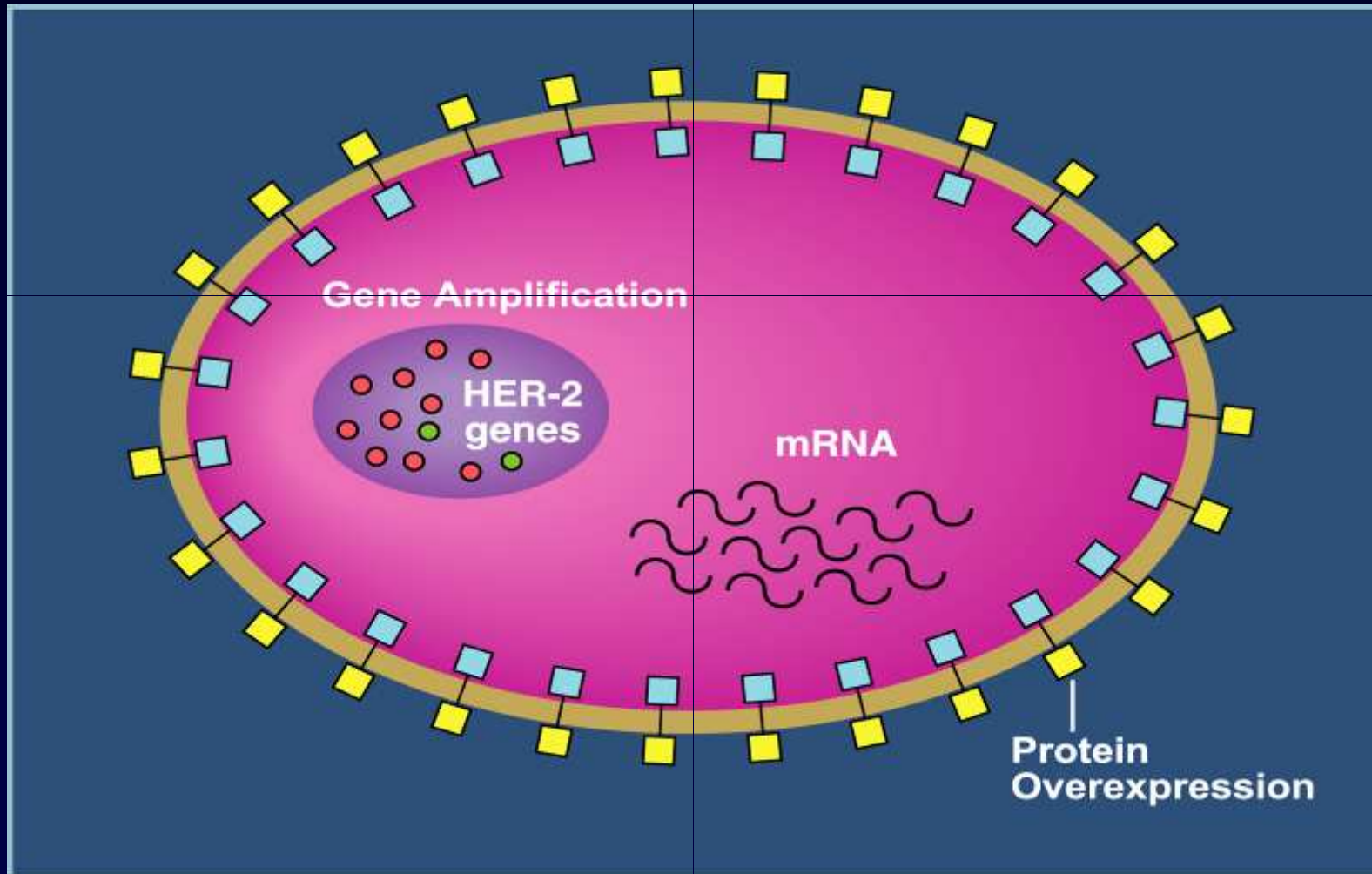
- HER2 is recognized as an important predictive and prognostic factor¹⁻³
- HER2 overexpression continues throughout the course of the disease and drives tumor growth⁴
- HER2 positivity is required for consideration of HER2-targeted Herceptin® (trastuzumab) and Lapatinib (Tykerb) therapy⁵

1. Witton et al. *J Pathol*. 2003;200:290; 2. Ross et al. *Oncologist*. 2003;8:307;
3. Konecny et al. *Clin Cancer Res*. 2004;10:1706; 4. Simon et al. *J Natl Cancer Inst*. 2001;93:1141;
5. Herceptin® (trastuzumab) PI, February 2005.

Testing Issues

- ◆ Integrity of the macromolecule being analyzed - degradation of DNA, RNA, or protein
- ◆ Accuracy of the reagent - variability of the antibodies
- ◆ Stability of the target, e.g. fixation artifacts in proteins - altering antigenic sites and recognition that the pre-analytic phase cannot be controlled
- ◆ Accuracy of the testing method
- ◆ Heterogeneity of the sample being tested

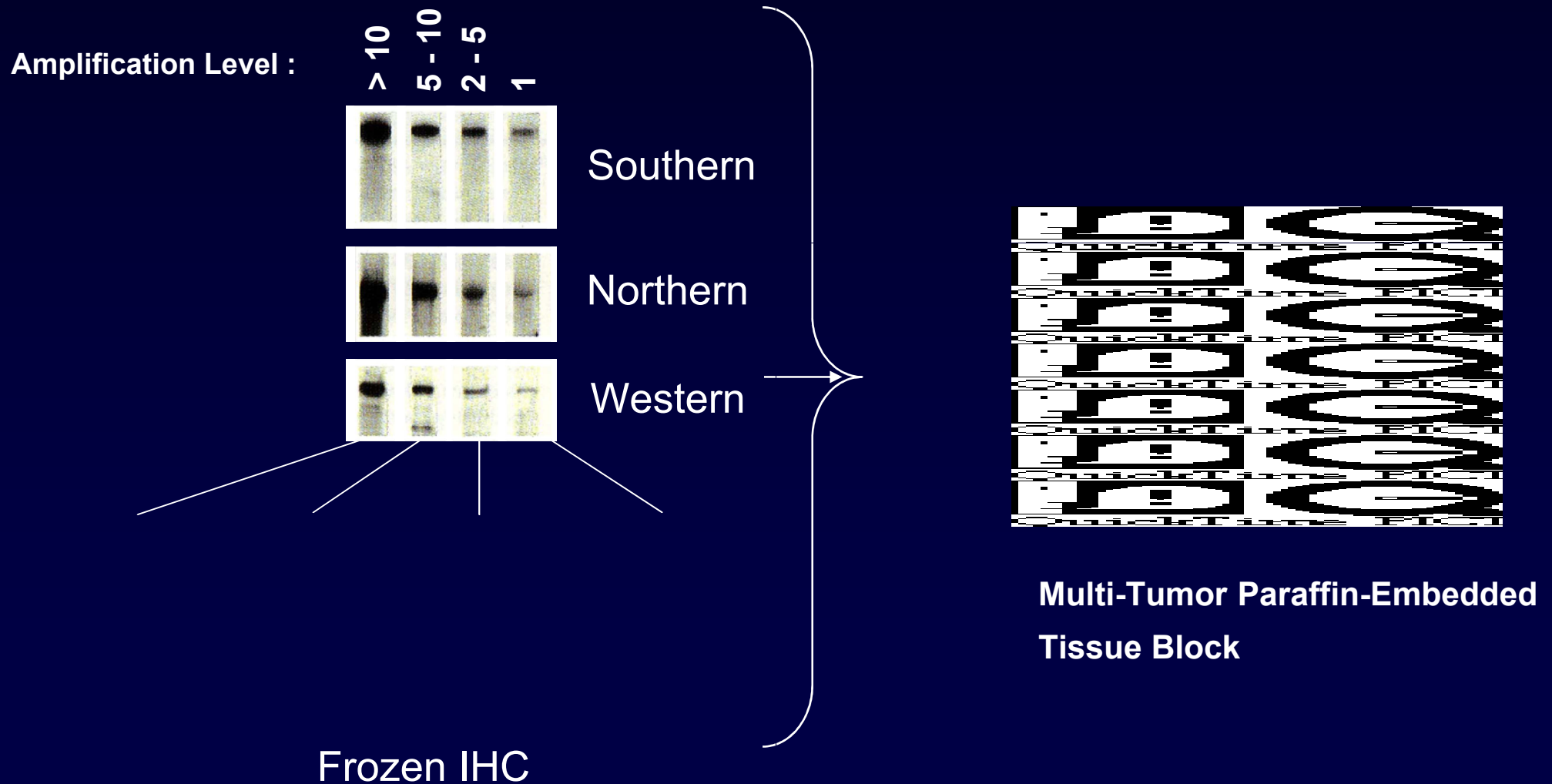
HER-2 Gene Amplification is Responsible for “Pathologic/Pathogenic” Overexpression



Molecularly Characterized Cohort

- ◆ A cohort of 189 snap-frozen breast cancer specimens of sufficient size to allow the simultaneous extraction of DNA, RNA and protein - all from the same specimen
- ◆ **Confirmed intact integrity** of the DNA, RNA and protein - I.e. no degradation of the macromolecules PRIOR to commencing analyses
- ◆ Formalin-fixed/paraffin-embedded tissue available from the exact same specimens
- ◆ Serves as the “**REFERENCE COHORT**” for all of our subsequent studies

HER-2 Molecularly Characterized Samples “REFERENCE COHORT” assembled in Multi-Tumor Blocks



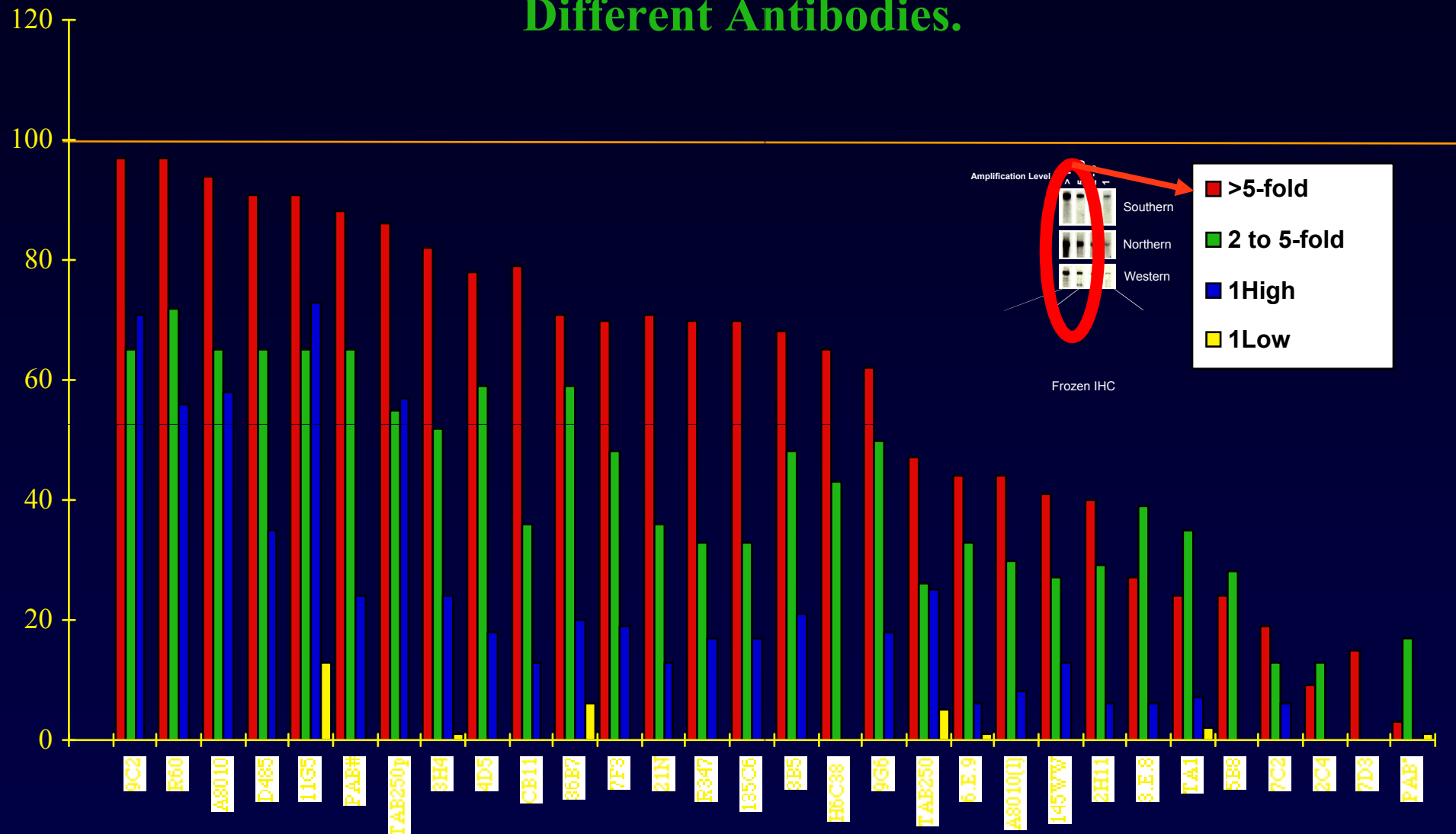
Testing Issues

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Testing Issues

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Percent of Breast Cancers in Various Expression Categories Identified by Immunostaining with 28 Different Antibodies.

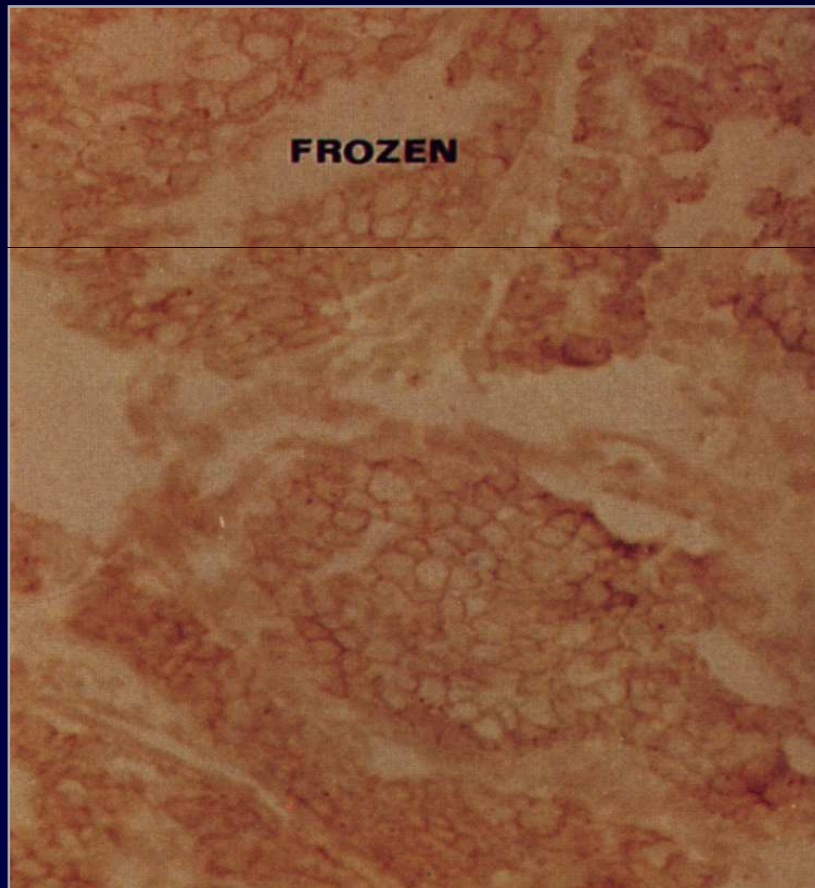


Testing Issues

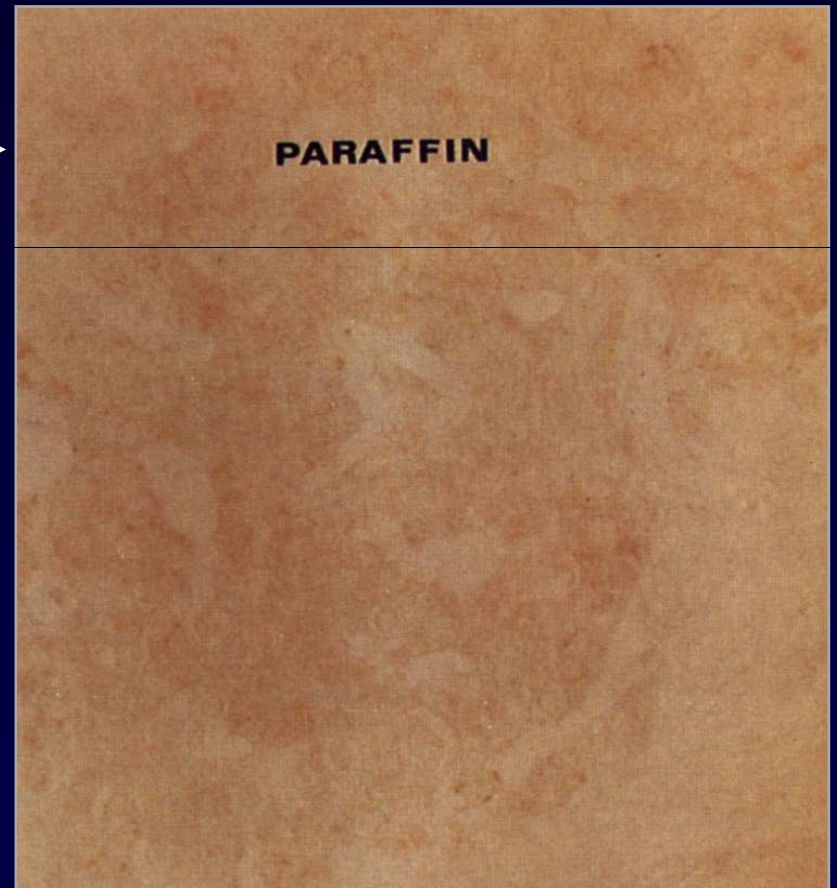
- ◆ Integrity of the macromolecule being analyzed - degradation of DNA, RNA, or protein
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- ◆ Stability of the target, e.g. fixation artifacts in proteins - altering antigenic sites and recognition that the pre-analytic phase cannot be controlled
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- ◆ Heterogeneity of the sample being tested

Fixation and Paraffin Embedding Result in Decreased Antigenicity

2 to 5-fold Amplified with Overexpressed

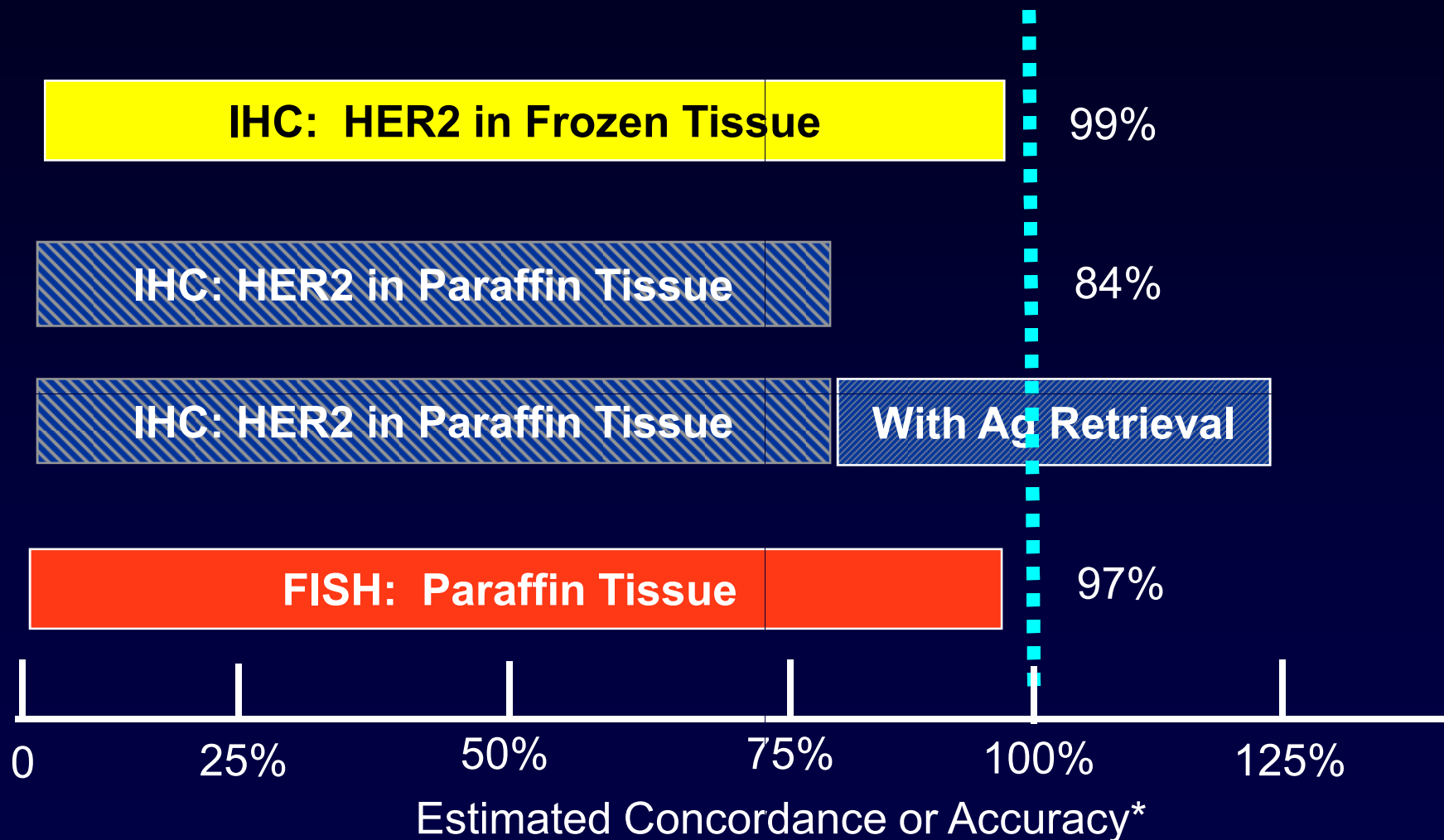


2 to 5-fold Amplified and Overexpressed



Enter - “Antigen Retrieval”

Schematic Summary of HER-2 Assay Results: Concordance with Known HER2-Positive Status “REFERENCE COHORT”

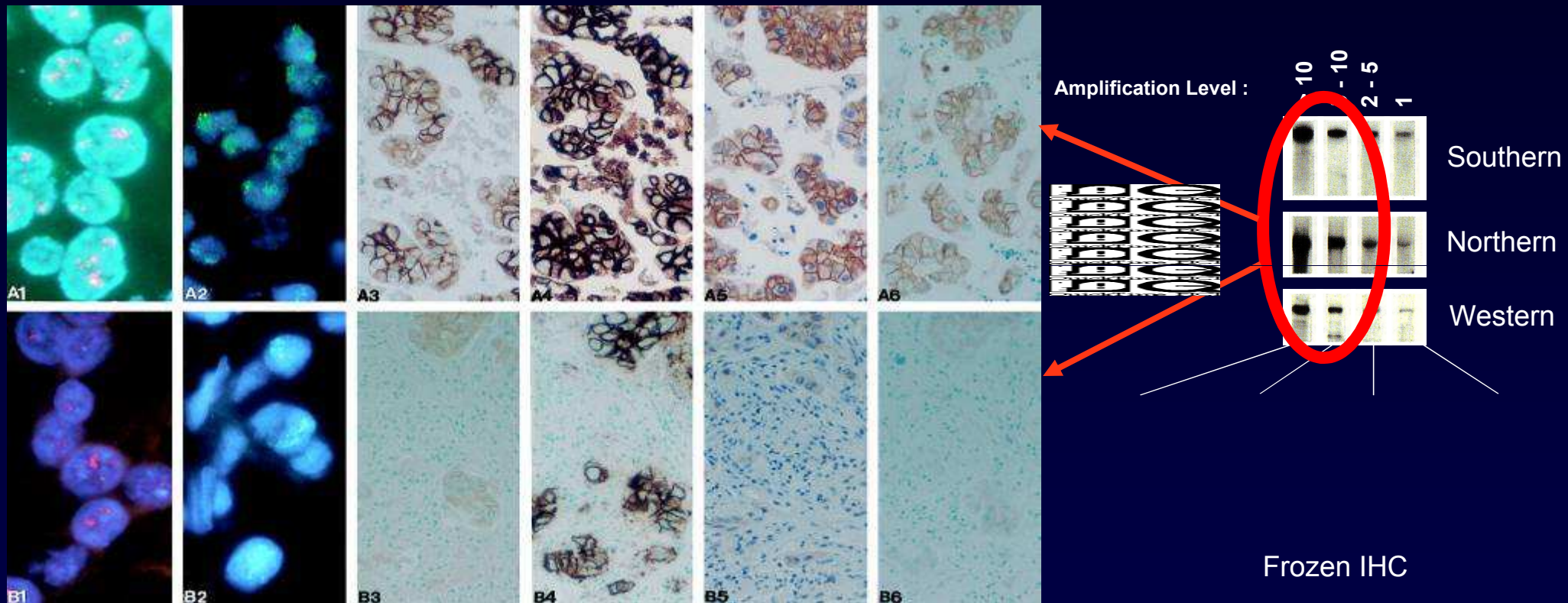


*Based on Results from *Science*, 1989; *Cancer Res.*, 1993; *Cancer Res.*, 1994; *Journal of Clinical Oncology*, 1997; *Journal of Clinical Oncology*, 2002; *Clinical Cancer Res.*, 2005.

Testing Issues

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Comparison of Six Different HER-2 Assays in Molecularly Characterized “REFERENCE COHORT” Breast Cancers Specimens



Press et al., *Journal of Clinical Oncology* 20: 3095-3105, 2002.

Comparison of FISH vs. IHC

Concordance Study: Two things to note

Results
1:1 population

		CTA-IHC			
		0	1+	2+	3+
FISH	-	207	28	67	21
	+	7	2	21	176
Amplification rate		3%	7%	24%	89%

3.7%

Overall concordance between FISH and IHC results was 82% (95% CI; 78–85%) ($p < 0.0004$).

Breast Cancer Research and Treatment 93: 3-11, 2005.

BCIRG Central Laboratory Concordance Study

Results

Results

		Local IHC				
		0	1+	2+	3+	
Central FISH	-	538	230	67	90	73%
	+	20	15	33	316	23%
Amplification rate		4%	6%	17%	78%	

4.3% N = 1407

Overall concordance between FISH and IHC results was 79% (95% CI; 77–81%).
Press et al., *Clinical Cancer Research*, 11: 6598-6607, 2005.

Arguments Against Screening with IHC and Reflex Testing with FISH

- ◆ Between 9 - 17% of women with HER-2/*neu* alteration are IHC-negative (0/1+) : definite false negatives.
- ◆ Between 8 and 22% of women with IHC 3+ do not have the HER-2/*neu* alteration (gene amplification by FISH) : ? false positives.
- ◆ Trastuzumab (Herceptin) and lapatinib are expensive therapeutics; errors in testing are costly.
- ◆ Women deserve the most accurate testing methods.

Response Rates in the Genentech H0649 Pivotal Clinical Trial of Trastuzumab

<u>FISH Ratio</u>	<u>Non-Resp (n)</u>	<u>Responder (n)</u>	<u>Rate (%)</u>	<u>95% CI</u>
<2.0	36	0	0%*	0%, 10%
2.0 - 6.0	75	11	13%*#	7%, 22%
>6.0	65	22	25% #	17%, 36%

FISH results obtained for 209 of the 222 (94%) women entered in trial.

Fisher's exact test, overall $p=0.0005$; * $p=0.033$, # $p=0.052$.

Response Rates in the Genentech H0650 Clinical Trial of Trastuzumab

<u>FISH Ratio</u>	<u>Non-Resp (n)</u>	<u>Responder (n)</u>	<u>Rate (%)</u>	<u>95% CI</u>
<2.0	28	1	3%*	0.1%, 18%
2.0 - 6.0	24	10	29%*#	15%, 47%
>6.0	31	18	37% #	23%, 52%

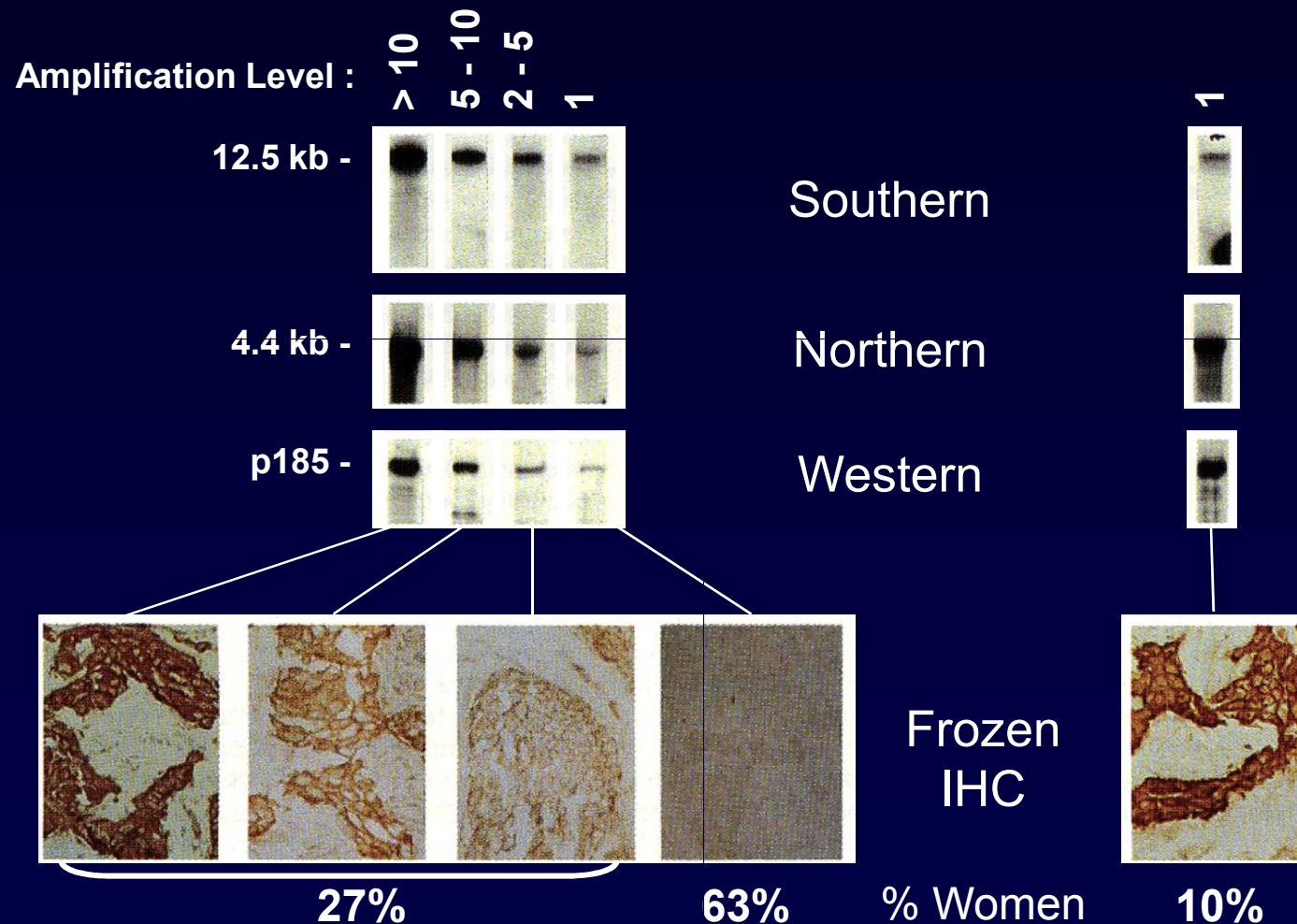
FISH results obtained for 112 of the 114 (98%) women entered in trial.

Fisher's exact test: overall p-value = 0.002; *p=0.008, #p=0.64.

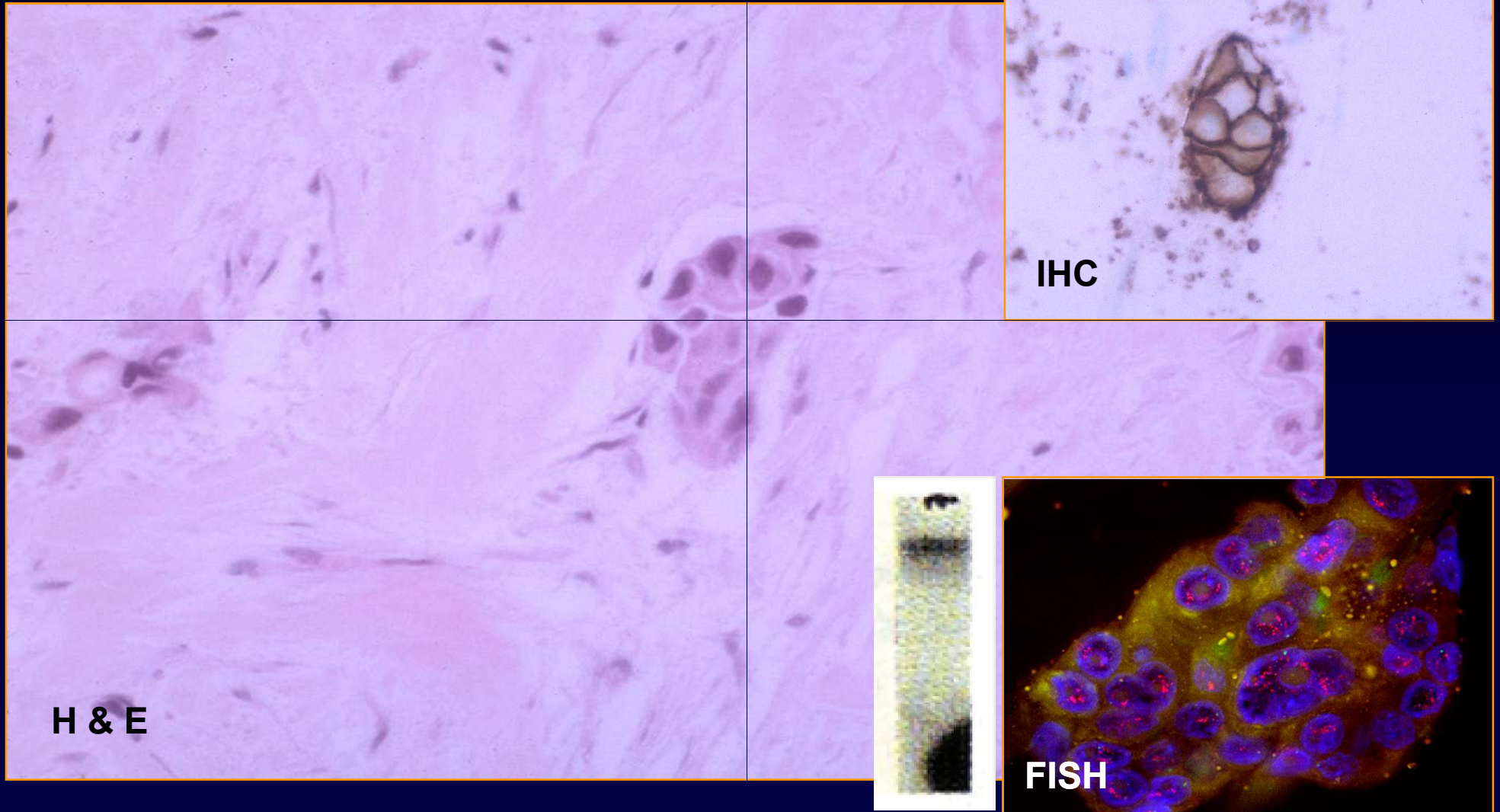
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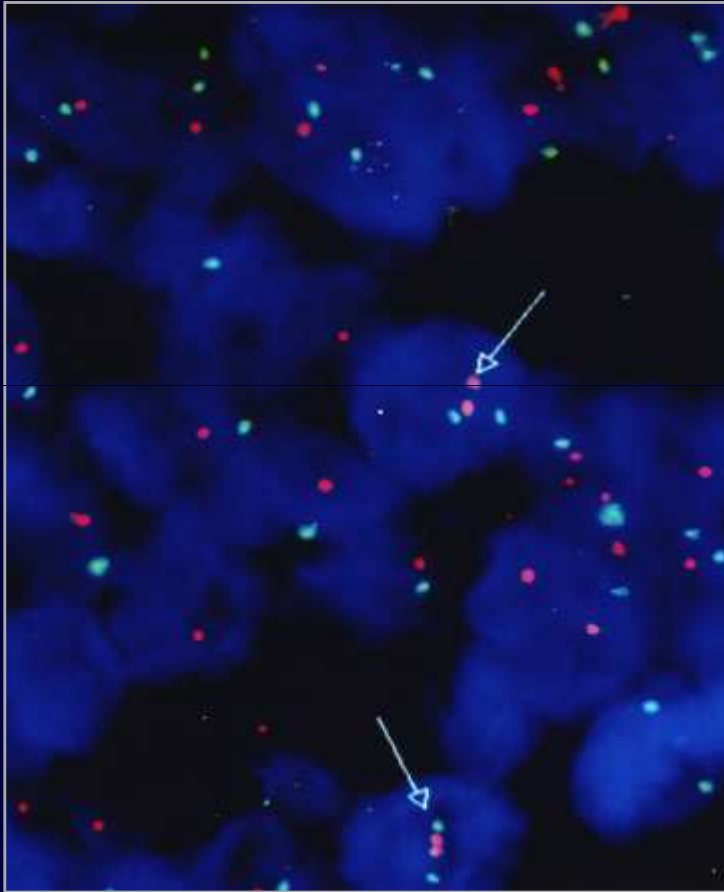
Correlation of HER-2 Gene Amplification with Overexpression



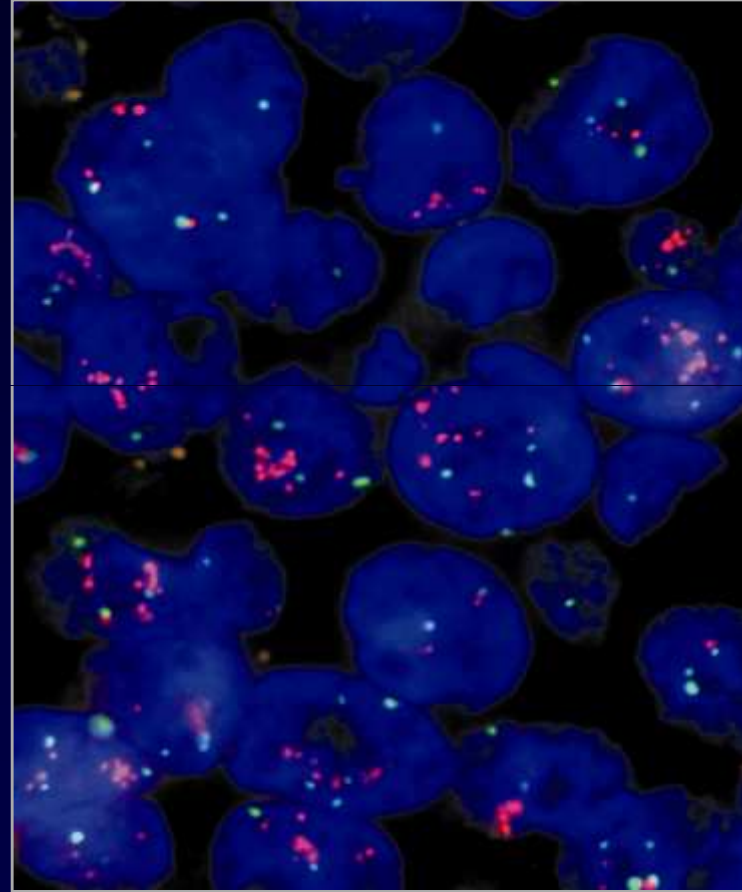
“Single Copy” Overexpression



HER-2 Gene Assessment by FISH



< 2.0 Not Amplified
(FISH-)

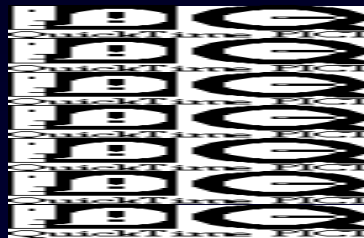


≥ 2.0 Amplified
(FISH+)

**Comparison: Solid Matrix Blotting Methods (frozen tissues) REFERENCE COHORT
with Fluorescence In Situ Hybridization (FISH) (paraffin-embedded tissues)**

Results

Gene Amplification by
Southern or Dot blot Hybridization



FISH

Pos (+)

Neg (-)

	Ampl	Not Ampl
Pos (+)	49	0
Neg (-)	1	90

N = 140

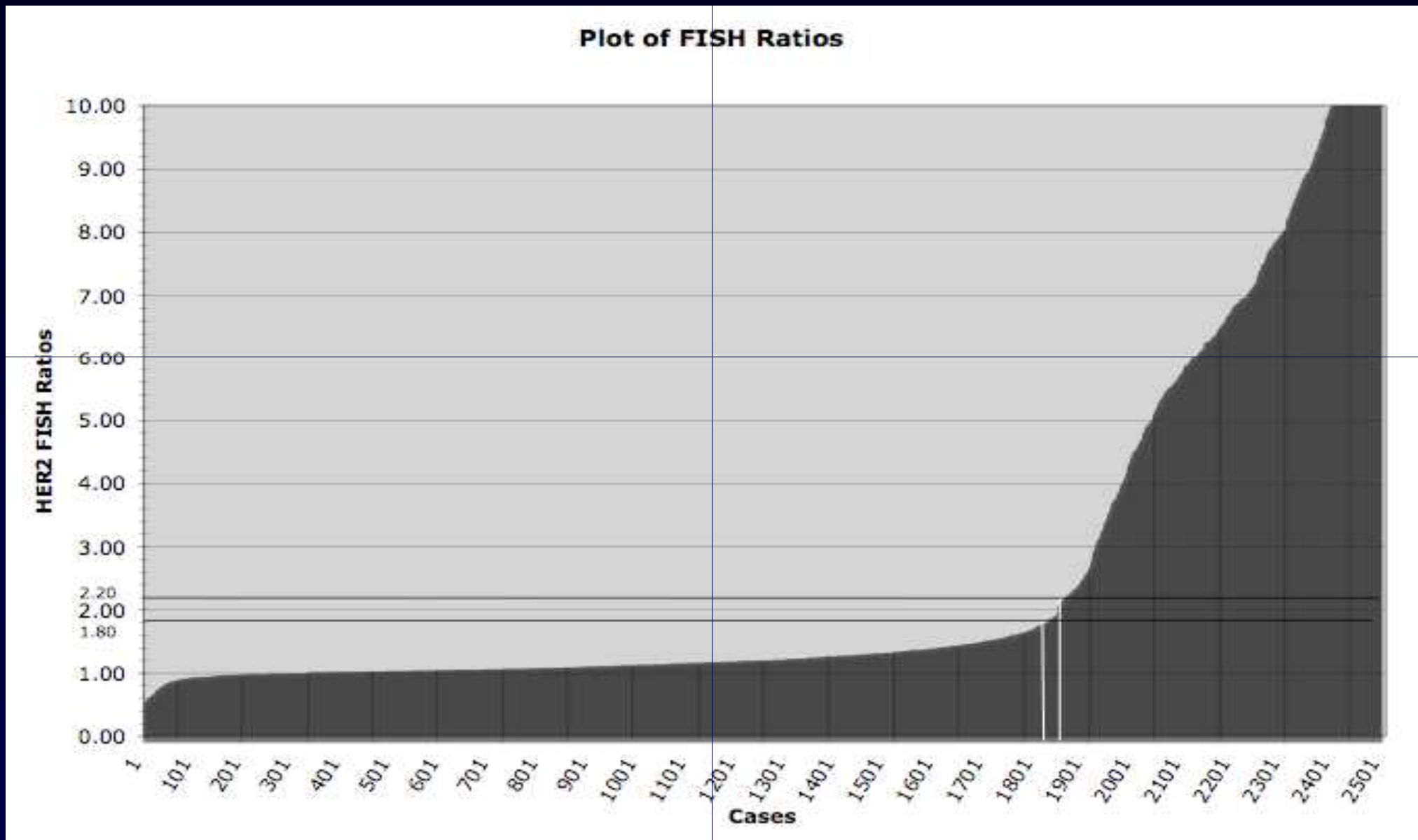
Sensitivity = 98%, Specificity = 100%.

Press et al., Journal of Clinical Oncology 15:2894-2904, 1997.

ASCO/CAP Guidelines

- ◆ New guidelines - A case is indeterminate i.e. may be called amplified, normal or equivocal if the ratio is between 1.8 -2.2 instead of the FDA approved definition of >2.0 = amplified.
- ◆ HAVE THEY DISCOVERED NEW FUNDAMENTAL BIOLOGY SINCE WATSON & CRICK OR THE KNOWN FIDELITY OF DNA REPLICATION DURING THE CELL CYCLE ???
- ◆ The consequence of this change has **not** been to make things easier. Instead, non-amplified cases are now sometimes called amplified. Conversely amplified cases may now be called non-amplified and hence either not receive the drug or incorrectly be classified as negative cases which benefit from trastuzumab or lapatinib

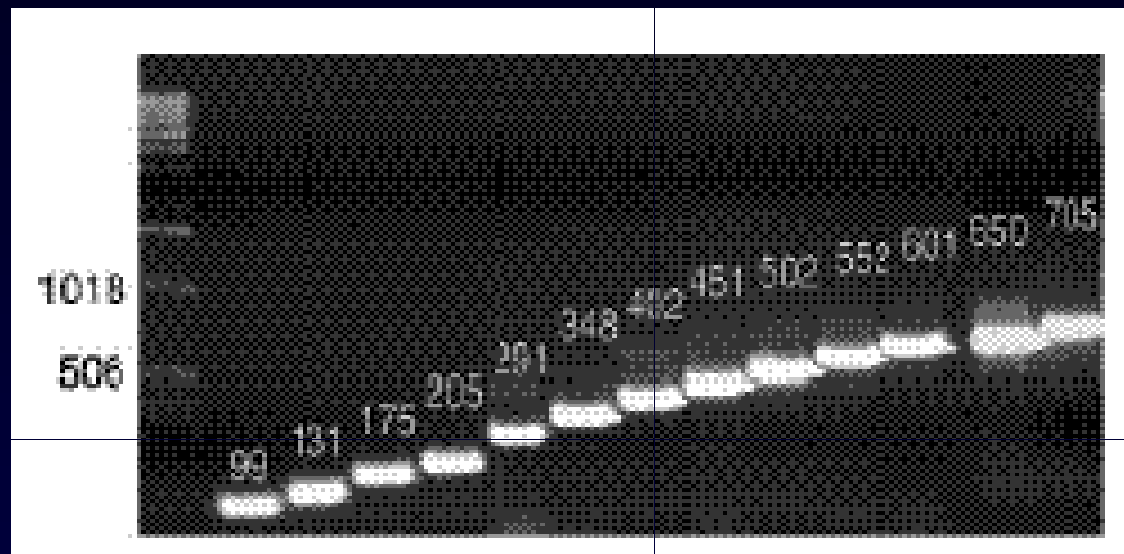
FISH Ratios Plotted from Lowest to Highest in the BCIRG Trials

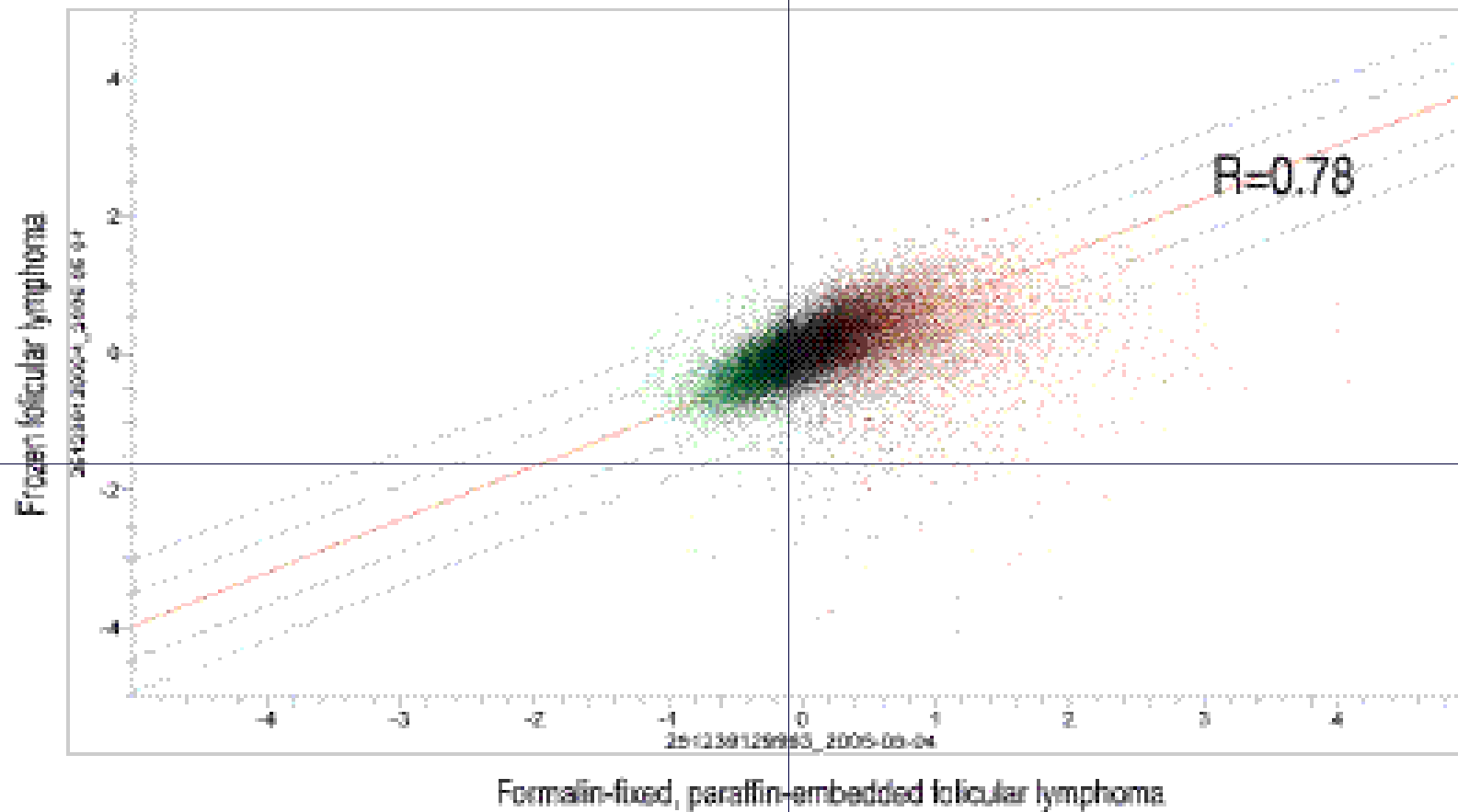


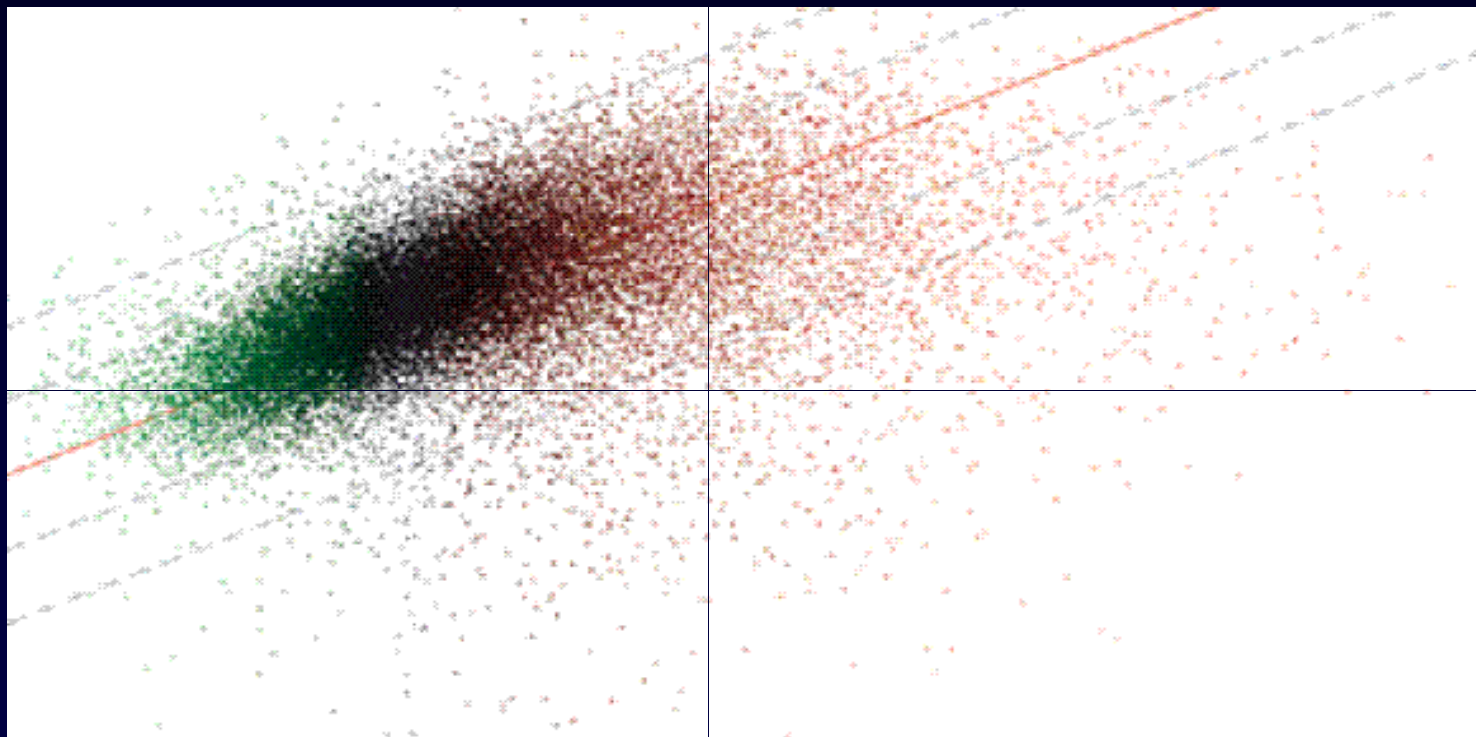
Use of Fixed/Paraffin Embedded Tissues for m-RNA Expression Levels

Testing Issues

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- ◆ Accuracy of the testing method
- ◆ Heterogeneity of the sample being tested







Chen, J et al. Diagn Mol Path 2007

Acknowledgements (con't)

- ◆ Genentech:

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Bob Mass,
Mark Sliwkowski

- ◆ Amgen:

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Elena Cajulis

- ◆ Nat. Br. Ca. Coalition

- ◆ USC:

Michael Press

- ◆ Revlon Foundation:

Ronald Perlman
James Conroy
Lilly Tartikoff

- ◆ Herceptin Clinical Investigators
Network & BCIRG

- ◆ Community-based/UCLA
Clinical Research Network

- ◆ The Group of 20